PHYTOREMEDIATION STUDY
FOR THE SANTA SUSANA FIELD LABORATORY
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Santa Susana Field Laboratory Soil Treatability Studies

Task III. Phytoremediation Study

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Environmental Remediation Services for Environmental Compliance for Area IV

Yarrow M. Nelson, Professor

Matthew Poltorak, Graduate Research Assistant

Michael Curto, and Peter Waldburger, Research Assoc. (Biology)

Alli Koivunen and Devon Dowd, Student Research Assistants

Department of Civil and Environmental Engineering

California Polytechnic State University

San Luis Obispo, CA 93407

Executive Summary

This phytoremediation study was one of five soil treatability studies commissioned by the US Department of Energy (DOE) as part of a larger remediation effort for Area IV of the Santa Susana Field Laboratory (SSFL); Area IV is referred to as "the site" in this document. Collectively, the purpose of these studies is to support the evaluation of methods for reducing the volume of contaminated soils that may need to be removed from Area IV by excavation, hauling, and disposal methods. Phytoremediation is the use of plants to contain or remove pollutants from the environment, or render them harmless through one or more biological mechanisms. The purpose of this phytoremediation study was to determine the potential for using plants native to the site to remediate the soil contaminants of interest (COIs), which include petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated dioxins/furans, mercury and silver.

This study was conducted in two phases. In Phase I, native and naturalized plants were collected from the site and analyzed for contaminant uptake as a screening tool to identify the best candidate species for further phytoremediation research. In Phase II, the best candidate plants were grown in controlled greenhouse experiments to determine remediation rates of phytoremediation and possible mechanisms of contaminant removal and/or biodegradation by the plants.

Over 30 plant species were considered for Phase I screening, but this list was narrowed down to nine species based on properties of the plants thought to make them suitable for phytoremediation and the availability of specimens growing in contaminated soils. The nine plant species screened were:

- *Nassella pulchra* Purple Needlegrass
- *Sambucus nigra* Blue Elderberry
- *Malosma laurina* Laurel Sumac
- *Baccharis salicifolia* Mule Fat
- *Ericameria palmeri* Palmer's Goldenbush
- *Hirschfeldia incana* Summer Mustard
- *Asclepias fascicularis* Narrowleaf Milkweed
- *Baccharis pilularis* Coyote Brush
- *Eriodictyon crassifolium* Thickleaf Yerba Santa

Three samples of each species growing in contaminated soil and one of each species growing in uncontaminated soil were selected for harvesting and analysis. The roots, aboveground plant tissue, and soil around the roots were sampled separately and analyzed for the COIs: PHCs, PAHs, PCBs, chlorinated dioxins/furans, and metals (which include mercury, silver, cadmium, and lead).

All of the plants in the field screening appeared to produce plant-based compounds that interfered with the total petroleum hydrocarbon (TPH) analysis, so it was not possible to ascertain uptake of petroleum hydrocarbons. PAH uptake by roots of several species was observed, with the highest PAH concentrations observed in the roots of Blue Elderberry

(1,740 milligrams per kilogram [µg/kg]), Purple Needlegrass (700 µg/kg), and Yerba Santa (200 µg/kg) (note µg/kg is also parts per billion (ppb) by weight). No uptake of PCBs was observed in the roots or foliage of any species. Chlorinated dioxins/furans were observed in the roots and foliage of several species, with the highest concentrations in the roots of Purple Needlegrass (2.2 µg/kg), Blue Elderberry (1.03 µg/kg), Palmer's Goldenbush (0.43 µg/kg), and Yerba Santa (0.42 µg/kg), and the highest concentrations in the foliage of Yerba Santa (0.90 µg/kg), Palmer's Goldenbush (0.76 µg/kg), and Purple Needlegrass (0.69 µg/kg). No uptake of mercury was observed in the roots or foliage of any species tested in the field. Silver uptake was observed in the roots of Laurel Sumac (7,300 µg/kg) and in the foliage of Summer Mustard (410 µg/kg).

For the Phase II greenhouse experiments, three plant species with a wide variety of observed contaminant uptake in the field were selected: Coyote Brush, Mule Fat, and Purple Needlegrass. Soil was collected from Area IV and these three species were grown in this soil under controlled conditions for seven months to quantify the removal of contaminants from the soil. Each planted microcosm consisted of 2.17 kg of soil in 4-L glass jars with glass marbles under the soil to allow for aeration. Plants were watered with deionized water and no leachate was removed from the soils. Five replicates of each microcosm type were created and incubated for 211 days with sampling of the soil initially and after 85 and 211 days. Plant roots and foliage were also analyzed for the COIs to determine the potential mechanisms of phytoremediation. One set of microcosms was used to test the effect of addition of a chelating agent (ethylenediaminetetraacetic acid, EDTA) and another set was used to test the effect of fertilizer addition on phytoremediation potential. Three control treatments were tested: sterilized (gamma irradiation) soil planted with Purple Needlegrass, unplanted soil, and sterilized unplanted soil.

Petroleum hydrocarbon concentrations in the soil (measured as extractable fuel hydrocarbons, EFH) did not decrease appreciably in the soil of any of the treatments over the first 85 days of plant growth. EFH soil concentrations at 211 days appeared to increase by a factor of five for all treatments, but this is likely an anomaly because these samples were analyzed by a different laboratory with different methods of quantifying EFH. The EFH analysis used for this study may have inadvertently included natural organic material (NOM), and further studies are underway at Cal Poly to quantify the contribution of such NOM to the EFH concentrations determined for site soils.

No changes in PAH concentrations were observed in the soil over the 211-day experiment. Reductions of PCB concentrations were observed in the soil of microcosms planted with Purple Needlegrass (49.4%) and chelated Coyote Brush (51.4%). However, the PCB concentrations in the soil of sterilized unplanted controls also decreased by 36.6% ($p < 0.05$). None of the species appeared to phytoextract PCBs into roots or foliage, but the mechanism of PCB remediation could be phytostimulation of rhizosphere microorganisms. PCB may have adsorbed to the glass in the microcosm jars, as indicated by the similar reductions in PCB concentrations for the sterilized, unplanted controls.

Purple Needlegrass showed the greatest uptake of dioxins/furans into the foliage but did not appear to reduce the dioxin/furan concentrations in the soil. Coyote Brush, fertilized Coyote Brush, and Mule Fat also showed uptake of dioxins/furans into the roots and foliage. Only the Coyote Brush and fertilized Coyote Brush significantly ($p = 0.036$, $p = 0.022$) reduced the total dioxin/furan concentration in the soil (17.8% and 19.8% respectively). Coyote Brush may have taken dioxin/furans into plant tissue and phytodegraded these compounds, or they may have stimulated microbes in the rhizosphere to better biodegrade the dioxins/furans.

None of the plants were identified as hyper-accumulators of metals, and none of the soil metal concentrations significantly decreased in any of the microcosms. All of the metals tested (except mercury) were taken into the roots of plants to some degree, with Purple Needlegrass showing the most promise for metal extraction as it showed some of the highest concentrations of metals in roots and was the only species that contained mercury and silver in the foliage.

Volatilization of COIs was tested for by collecting and trapping vapors from the plants over the course of 5 days. No volatilization of any of the COIs was observed under these conditions for any of the plant species. Volatilization of mercury was not tested for.

This study suggests that phytoremediation of the organic COIs at the site will proceed slowly at best. There appears to be some potential for phytoremediation of PCBs and chlorinated dioxins/furans indicated by the greenhouse microcosm experiment. However, it is likely that the soil contaminants at the site have been highly degraded through 20-50 years of natural processes in the field, and these processes are likely to have decreased the bioavailability of the contaminants to plants and/or soil microbes. Further, the easily biodegraded compounds have likely biodegraded years ago leaving the more recalcitrant compounds (either original compounds or degradation products) in the soil at present. Metal uptake was also not substantial enough to lower metal concentrations in the soils. Thus phytoremediation of COIs at the site is limited and more aggressive forms of remediation may be required to reduce the concentrations of COIs in a more reasonable time period. Phytoremediation could be employed for portions of the site with low COI concentrations where the length of time required for phytoremediation would not be an issue. Planting with native plants could likely be a part of site restoration efforts, and provide long-term enhancements to other shorter-term COI remediation efforts.

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1.0. Introduction and Scope

The purpose of this study was to determine if phytoremediation could be used to reduce contaminant concentrations in the soil in Area IV of the Santa Susana Field Laboratory (SSFL); Area IV is also referred to as "the site" in this document. Phytoremediation is the use of plants to contain or remove pollutants from the environment, or render them harmless through one or more biological mechanisms (Cunningham and Berti 1993; Salt, Smith, and Raskin 1998; Pilon-Smits 2005). Phytoremediation provides an *in-situ* alternative to more aggressive and intrusive forms of conventional remediation (EPA 1999). When compared to excavation and other physical/chemical remediation methods, phytoremediation is less expensive and provides several additional benefits, including (1) contaminant containment, (2) possible extraction of metals with market value, and (3) durable land management that can gradually improve soil quality (Vangronsveld et al. 2009; Aken, Correa, and Schnoor 2010). The absence of energy-consuming equipment and limited maintenance, little or no negative environmental impacts, and public acceptance as a "green technology" are also important advantages of phytoremediation (Gerhardt et al. 2009).

The SSFL was established in 1947 by North American Aviation for testing liquid-propulsion rocket engines. SSFL was divided into four different areas, and the Department of Energy (DOE) performed research in a section of Area IV named the Energy Technology Engineering Center (ETEC). During the ETEC's operation, the soil was contaminated with petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated dioxins/furans, and heavy metals which together are referred to as the contaminants of interest (COIs). After the closure of ETEC, the DOE was responsible for the cleanup of soil in Area IV. The DOE commissioned this study of phytoremediation as one of five soil treatability studies that were designed to support the evaluation of methods for reducing the volume of contaminated soils that may need to be removed from Area IV by traditional excavation, hauling, and disposal methods (Sandia National Laboratories 2012).

Most prior phytoremediation research has focused on a single contaminant (Blaylock et al. 1997; Campanella, Bock, and Schröder 2002; Cook and Hesterberg 2013; Cordale Johnson, John Thomlinson 2009; D'Orazio, Ghanem, and Senesi 2013; Duckart, Waldron, and Donner 1992; Ficko, Rutter, and Zeeb 2010; Newman et al. 1997; Wei et al. 2009). However, the site contains multiple contaminants, and thus phytoremediation needed to be investigated with the specific multiple soil contaminants found at the site. It is also desirable to use plants native to the site, and little research has been done on phytoremediation with the native plants growing at the site. If native plant species indigenous to the site were found to be useful for phytoremediation, ecological restoration could be accomplished simultaneously with phytoremediation through revegetation efforts.

The specific objectives of this study were to determine what plant species are presently growing in Area IV soils that may be contributing to phytoremediation, what are the phytoremediation mechanisms for contaminant uptake/degradation, and what

nutrients/additives can be added to stimulate/increase phytoremediation rates (Sandia National Laboratories 2012). This study was conducted in two phases – the first phase to identify candidate species based on field studies at SSFL of contaminant uptake, and the second phase growing plants under controlled greenhouse conditions to quantitatively examine phytoremediation by the best candidate species.

In Phase I of this study, native and naturalized plants growing in the contaminated areas at SSFL were harvested and analyzed to assess their phytoremediation potential. Uptake of COIs was considered an indicator of phytoremediation potential, so uptake of COIs by plants currently growing in the contaminated soil was used to screen for the best candidates for further study.

In Phase II of the study, three of the most promising species were grown in greenhouse microcosms to quantify the removal of contaminants from the soil. Several different microcosm treatments and controls were used to elucidate the effectiveness of phytoremediation. A chelating agent ethylenediaminetetraacetic acid (EDTA) was added to one set of microcosms and fertilizer was added to another set to test their effect(s) on the remediation process. There were three control microcosm sets: unplanted, sterilized planted, and unplanted sterilized. The COI concentration in the soil was measured at 0, 85, and 211 days after planting. Both the roots and foliage from the plants were tested for COIs at the end of the 6-month experiment. Emissions from the microcosms were sampled with sorbent tubes and measured to identify any COI volatilization from the plants.

2.0. Background and Literature Review

2.1. Phytoremediation Overview

Phytoremediation has applications in many sites where there are chlorinated solvents, fuel spills, ammunition wastes, landfill leachates, and agricultural runoff (Schnoor et al. 1995). Phytoremediation is typically used to remediate areas where soil contamination is shallow and accessible by the plant roots. Plants can often survive higher pollutant concentrations than many microorganisms that are used for bioremediation (Schnoor et al. 1995). Phytoremediation can be used to remediate many different contaminants because of the different phytoremediation mechanisms that are used in the phytoremediation process. These mechanisms coupled with the unique characteristics of individual plant species can be a formidable remediation option for contaminated media. The following sub-sections give detailed descriptions of each phytoremediation mechanism and the pollutants that are most affected by it.

2.2. Mechanisms of Phytoremediation

Water, slurry, and soil matrices can be remediated through the use of various plant mechanisms listed by Salt, Smith, and Raskin (1998):

- ***Phytoextraction:*** the use of pollutant-accumulating plants to remove metals or organics from soil by concentrating them in the harvestable parts
- ***Phytodegradation:*** the use of plants and associated microorganisms to degrade organic pollutants;
- ***Rhizodgradation:*** the use of associated microorganisms to degrade organic pollutants in the root-soil zone;
- ***Rhizofiltration:*** the use of plant roots to absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams;
- ***Phytostabilization:*** the use of plants to reduce the bioavailability of pollutants in the environment;
- ***Phytovolatilization:*** the use of plants to volatilize pollutants

All of these mechanisms of phytoremediation are depicted in Figure 2.1 and described in more detail below.

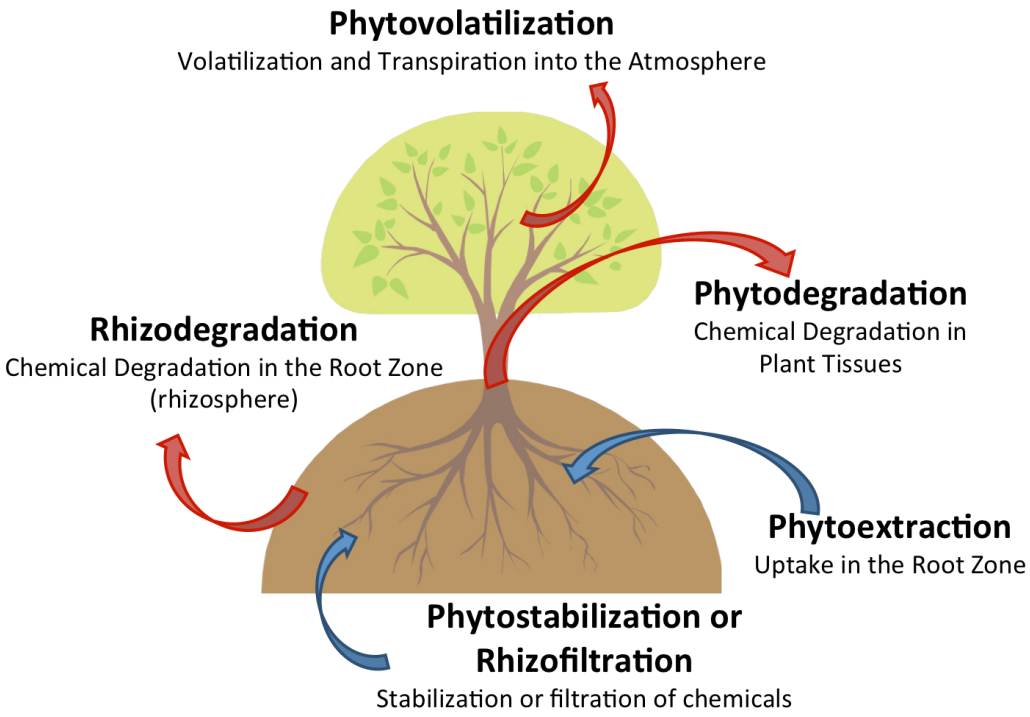


Figure 2.1. The Six Mechanisms of Phytoremediation

2.2.1. Phytoextraction

Phytoextraction is the mechanism that extracts contaminants from the surroundings and transports them in the roots, stem, or foliage part of the plant (Greenwood, Rutter, and Zeeb 2011). Both organic and inorganic contaminants can be extracted by plants (Newman et al. 1997; Slater, Gouin, and Leigh 2011; Huelster, Mueller, and Marschner 1994; Jianwei W. Huang et al. 1997; McGrath and Zhao 2003), however, this mechanism is particularly suited for the remediation of heavy metals from the environment since only organics with a log K_{ow} (octanol-water partitioning coefficient) between 0.5-3.0 are typically able to be extracted from soil/water (Schnoor et al. 1995). There are several possible defense mechanisms that plants may use to tolerate heavy metals and the primary mechanism is chelation using binding proteins such as metallothioneins or phytochelatins (Mejáre and Bülow 2001). These proteins can bind to metals and form a complex, thereby increasing the bioavailability of the contaminant which can then be more readily taken up into the plant. Thus the effectiveness of phytoextraction can be largely based on the bioavailability of the contaminants in the soil. Lowering pH, using soil microorganisms that stimulate metal uptake, and adding chelating agents to soil can often greatly increase the bioavailability of metals (Jianwei W. Huang et al. 1997; Salt, Smith, and Raskin 1998). Plants can be genetically engineered to express genes that will increase heavy metal tolerance or facilitate greater metal uptake than would normally occur. For example the *merA* gene encodes mercuric reductase which can reduce mercuric ions (Hg^{2+}) into the less toxic elemental mercury (Hg^{0}) which can be taken up into the plant and potentially volatilized into the atmosphere (Rugh et al. 1998; Wang et al. 2012).

If a plant accumulates metals in the foliage or stems, those sections can be harvested and the metals disposed of safely. The plant can then grow back, accumulate more metals, and be harvested until the surroundings have been remediated (McGrath and Zhao 2003). This process is particularly beneficial with certain plants that have been identified as metal hyperaccumulators. Although the exact criteria used to identify metal hyperaccumulators is under discussion (Ent et al. 2013), in general, metal hyperaccumulators are plants capable of storing metals in their tissue at concentrations that are much higher than the surrounding environment (Baker and Brooks 1989; Memon and Schröder 2009). With such high concentrations, it can be beneficial to extract and reuse the metals collected in the plant. Metal price, plant biomass, and the maximum metal concentration achievable in plant tissue are important factors in making metal extraction economically feasible (Brooks et al. 1998).

While metals may be the primary focus of phytoextraction, many different organic compounds can also be extracted. Alfalfa and other plant species have been shown by several studies to extract PCBs from soil or water (Zeeb et al. 2006; Ficko, Rutter, and Zeeb 2010; Xu et al. 2010; Ying Teng et al. 2010; Liu and Schnoor 2008; Greenwood, Rutter, and Zeeb 2011). Chlorinated dioxins can also be extracted from the environment using plants (Campanella and Paul 2000; Huelster, Mueller, and Marschner 1994).

2.2.2. Phytodegradation

Phytodegradation is the uptake and degradation of contaminants within plants, or the degradation of contaminants in soil or water using enzymes exuded by plants (J. H. Lee 2013; Pilon-Smits 2005; Gerhardt et al. 2009). Organic compounds which are introduced into the plant are metabolized in a similar process as contaminants in an animal liver; this is known as the "green-liver" concept (Shang, Newman, and Gordon 2003). Two sequential processes are used to phytodegrade contaminants. The first is chemical transformation by enzyme-catalyzed reactions which often results in a less toxic product; the second is compartmentation of the transformed contaminant into the vacuoles or apoplast of the plant (Coleman, Blake-Kalff, and Davies 1997). The enzymes inside the plant matrix can degrade organic contaminants into inorganic compounds like CO_2 or water, or degrade them partially into stable intermediates after they enter the plant (Pilon-Smits 2005). The presence of enzymes such as dehalogenase, nitroreductase, and peroxidase, catalyze the transformation of organics inside plant tissue (Schnoor et al. 1995; Salt, Smith, and Raskin 1998). Plants are capable of metabolizing a wide variety of persistent organic pollutants (POPs) like pesticides and PCBs (Aken, Correa, and Schnoor 2010). Phytodegradation has also been used on ammunition wastes such as trinitrotoluene (TNT) and the chlorinated organic compound trichloroethylene (TCE) (Schnoor et al. 1995). Since the organic compounds must first be phytoextracted by the plant, moderately hydrophobic contaminants with a log K_{ow} between 0.5-3.0 are well suited for phytodegradation (Schnoor et al. 1995). As a general trend, highly hydrophilic compounds (log $K_{ow} < 0.5$) have difficulty passing through the plant membranes, whereas highly hydrophobic compounds (log $K_{ow} > 3.0$) bind tightly to the roots and are not translocated well within the plant (Briggs, Bromilow, and Evans 1982; Aken, Correa, and

Schnoor 2010). This can limit the effectiveness of phytoremediation of some hydrocarbons because hydrocarbon compounds have log K_{ow} values that range from 0.37 to 6.57 (Heath et al., 1993). However, contaminants that are highly hydrophobic/hydrophilic are not necessarily excluded from phytodegradation consideration. Zucchini exudates have been shown to bind to dioxins creating a complex that decreases the log K_{ow} and improves the uptake of dioxin into the plant (Campanella, Bock, and Schröder 2002). Also, if the contaminants are transformed inside the plant, they could become more amenable to translocation and subsequent degradation. Examples of moderately hydrophobic contaminants are MTBE (methyl-tert-butyl ether) with a log K_{ow} of 1.2, BTEX (benzene, toluene, ethylbenzene, and xylene) with a log K_{ow} of 2.13, 2.69, 3.15, and 3.12-3.2 respectively (EPA 1995), chlorinated solvents, and short-chain aliphatic hydrocarbons (Newman and Reynolds 2004; Schnoor et al. 1995; J. H. Lee 2013). As stated previously, studies have shown the uptake of PCB's (Zeeb et al. 2006; Ficko, Rutter, and Zeeb 2010) and dioxins (Huelster, Mueller, and Marschner 1994) into plant tissue. But PCB metabolism in plants is slow and dioxins have not yet been shown to degrade within the plant (Campanella, Bock, and Schröder 2002).

2.2.3. Rhizodegradation

The rhizodegradation mechanism is also referred to as "rhizoremediation," "phytostimulation," or "rhizostimulation." The rhizosphere is the zone of the root-soil interface where there is increased microbial activity and biomass due to the effect of the plant roots. This mechanism uses the symbiotic relationship between plants and bacteria/fungi in the rhizosphere to enhance biodegradation of organic contaminants by stimulating the microbial community (Anderson, Guthrie, and Walton 1993). Several types of bacteria and fungi are capable of partially or completely degrading contaminants in the soil through the use of enzymes like dehalogenase or peroxidase (Gerhardt et al. 2009). Mycorrhizae fungi can grow in symbiotic association with plants and help degrade organics that are recalcitrant to bacteria alone (Schnoor et al. 1995). Plant root exudates can enhance the degradation of pollutants by stimulating the survival and action of these microbes present in the rhizosphere (Kuiper et al. 2004; Salt, Smith, and Raskin 1998). Certain bacteria such as *Pseudomonas putida* and *Azospirillum spp*. are even capable of coaxing nutrient release from plants using biochemical signals (Anderson, Guthrie, and Walton 1993).

Rhizoremediation can be used to degrade a wide variety of contaminants like BTEX, PAHs, and petroleum hydrocarbons using trees and grasses to stimulate the remediation (Cook and Hesterberg 2013). An 80-day greenhouse experiment showed the remediation of phenanthrene- and pyrene-contaminated soil using several plant species to stimulate the rhizosphere (S.-H. Lee et al. 2008). Similarly, increased bacterial and fungal counts were observed when pyrene soil concentrations were reduced in the laboratory using *Medicago sativa*, *Brassica napus*, and *Lolium perenne* plant species (D'Orazio, Ghanem, and Senesi 2013). Grasses are known to stimulate the rhizosphere and much of the phytoremediation research for removal of organic compounds such as petroleum hydrocarbons (PHCs) focuses on *Poaceae* grass species (Hall, Soole, and Bentham 2011).

Persistent organic pollutants (POPs) such as PCBs and dioxins can also be remediated by enhancing the rhizosphere. Campanella et al. (2002) list four ways plants aid in degrading chlorinated compounds: (1) Root exudates contain compounds readily available for bacterial metabolism, (2) Plants increase the rhizosphere oxygen content, (3) Plants produce pollutant analogs, and (4) Root exudates contain general growth-promoting factors. One study demonstrated PCB degradation enhancement using two grass species (*Phalaris arundinacea* and *Panicum virgatum*) to stimulate the rhizosphere and increase enzyme activity (Chekol, Vough, and Chaney 2004). Several studies have suggested that rhizoremediation was the primary mechanism of PCB degradation; stimulated enzyme activity and increased microbial population in the root zone supported this hypothesis (Li et al. 2013; Chekol, Vough, and Chaney 2004; Xu et al. 2010; Ying Teng et al. 2010). A study done on dioxin remediation by melon (*Cucumis melo*) and zucchini (*Cucurbita pepo L. var. Diament*) showed that certain compounds released by these plants bind to the contaminant and increase the hydrophilic nature and increase the bioavailability of dioxins (Campanella and Paul 2000). The increased bioavailability enables bacteria or fungi to better access the contaminants and degrade them more efficiently.

Sometimes the bacterial degradation of a contaminant will be halted if another compound is competing for degradation. For example, *Burkholderia xenovorans* LB400 can use PCBs as a carbon source, however, PAH compounds can act as a competing carbon source since bacteria in the *Burkholderia* genus are known to degrade PAHs (Seo, Keum, and Li 2009). The interaction between contaminants is important and studies are underway to identify other molecules that may interfere and compete with PCBs for bacterial degradation (Secher et al. 2013).

Although the research is promising, there are two primary challenges of applying rhizoremediation in the field that are listed by Gerhardt et al. (2009): (1) Plants in the field experience additional stresses that are not present in laboratory conditions, (2) Current methods of assessing rhizoremediation may not be sufficient to determine whether contaminant concentrations are decreasing or not. There are many differences between the field and the laboratory, for example, plants in the field will experience nutrient deficiencies and harsh weather that do not occur under laboratory conditions. Often the distribution of contaminants in soil is uneven and includes areas of extremely high concentrations or “hot spots” which differs from the generally well-mixed laboratory soil.

2.2.4. Rhizofiltration

Rhizofiltration is used to remediate aqueous waste streams by absorbing/adsorbing contaminants to plant roots thus preventing contaminants from traveling horizontally downstream or leaching into the ground (Dushenkov et al. 1995; Raskin, Smith, and Salt 1997). Since aqueous waste streams are not an aspect of concern for this project, rhizofiltration will not be discussed in detail.

2.2.5. Phytostabilization

Phytostabilization stabilizes or immobilizes contaminants in the soil using plants, which in turn reduces the bioavailability of pollutants in the environment (J. H. Lee 2013). This process sequesters the contaminants in the soil near the root zone, but does not degrade or absorb the contaminant into the plant tissue (Morikawa and Erkin 2003). Phytostabilization is a less-researched area of phytoremediation (Raskin, Smith, and Salt 1997), and since the ultimate objective of this project was to remove the contamination from SSFL Area IV, phytostabilization will not be discussed in detail.

2.2.6. Phytovolatilization

Phytovolatilization is a mechanism used to release contaminants from the plant into a gaseous form (Salt, Smith, and Raskin 1998; Pilon-Smits 2005). Phytovolatilization can occur after an absorbed pollutant is translocated to the foliage of the plant and volatilized. After observing the garlicky odor of certain plants, Lewis, Johnson, and Delwiche (1966) discovered that selenium compounds were being volatilized by both accumulator and non-accumulator species. Other studies have since confirmed those results and shown that certain plants can convert inorganic selenium into volatile forms such as dimethylselenide (Duckart, Waldron, and Donner 1992; Terry et al. 2000). Orchard et al. (2000) developed a novel laboratory system to show that TCE was being extracted and volatilized by plants grown in a hydroponic solution. Further studies have shown volatilization of TCE and other volatilize organic contaminants (VOCs) such as MTBE (Yu and Gu 2006; Newman et al. 1997).

Another promising area of phytovolatilization is the transformation of mercuric ions (Hg^{2+}) into the less toxic elemental mercury (Hg^{0}) which can be volatilized by specific plants. However, natural plants have not demonstrated enough Hg (total mercury) volatilization to be useful without some genetic modification (Heaton et al. 1998). The main barriers to Hg uptake are lack of Hg bioavailability and poor translocation of Hg from the roots to the aerial portion of the plant (Heaton et al. 1998). But certain plants, such as willows or tobacco, can be genetically altered to express the *merA* gene which enables a greater resistance to mercury contamination and a greater rate of Hg^{0} volatilization (Rugh et al. 1996; Rugh et al. 1998). *MerB* is another gene that encodes a mercury-processing enzyme called organomercurial lyase. This enzyme catalyzes the breaking of carbon-mercury bonds in methyl-mercury (MeHg) and produces Hg^{2+} which is then used by the mercuric reductase enzyme and volatilized (Heaton et al. 1998).

Chelating chemicals such as EDTA or sodium thiosulfate have been shown to increase the bioavailability of mercury in the soil with limited effect on the physical or chemical soil properties (Wang et al. 2012). The increased bioavailability of mercury may allow phytoextraction from the soil and increase the amount of phytovolatilization if the plant is capable. Phytovolatilization does not require harvesting for the contaminant elimination process and therefore has potential to be a powerful remediation tool (Pilon-Smits 2005). However, since the contaminants are released to the atmosphere, careful

analysis must be done to ensure that phytovolatilization does not create an air pollution problem.

An extensive list (from current available research) of the different plant species and methods used to investigate contaminant phytovolatilization is shown in Table 2.1.

2.3. Site History and Characterization

The Santa Susana Field Laboratory (SSFL) was established in 1947 by North American Aviation as a location for testing liquid-propulsion rocket engines. Testing was done initially for the Department of Defense and later for the National Aeronautic Space Administration (NASA). Area IV of SSFL was used for energy and liquid metals research from the mid-1950s until approximately 2000. A 90-acre portion of Area IV was leased to the Department of Energy (DOE) for nuclear energy and other research (Department of Energy 2003). This 90-acre portion of Area IV was termed the Energy Technology Engineering Center (ETEC) and also served as DOE's Liquid Metals Center of Excellence. Ten small nuclear reactors were tested during ETEC operations and a variety of chemicals were used during the operation of research in Area IV. These chemicals included PCBs used in electrical components and hydraulic fluids, fuels that ran auxiliary generators and heated water for steam, metals such as silver for photograph development, and mercury for cooling the nuclear reactors. Onsite waste burning and a wildfire in 2005 produced dioxins/furans, and releases of PCBs, metals, fuels, and lubricants contaminated the soil within Area IV. In addition, solvents from transformers, storage tanks, drums, and leach fields also contributed to contamination.

2.3.1. Soil Characteristics

The soil in Area IV varies depending on the sub-area. The sub-areas of Area IV that contain most of the contamination are primarily loamy soils, either Saugus sandy loam with 5 to 30 percent slopes, or Zamora loam with 2 to 15 percent slopes (HydroGeoLogic Inc. 2012). The soil is aerobic with measured O_2 concentrations ranging from 13% to 20% (June, 2014). The soil temperature in March 2014, when plant samples were collected, one foot below the ground surface ranged from 61-86 ^{o}F.

2.3.2. Soil Contamination

The contaminants of interest (COIs) at SSLF Area IV fall into five general categories:

- Petroleum hydrocarbons (measured as extractable fuel hydrocarbons [EFH] and quantitated for a range of alkanes C8-C11, C12-C14, C15-C20, C21-C30 and C30-C40)
- Polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs)
- Chlorinated dioxins/furans
- Heavy metals

Table 2.1. Phytovolatilization experimental methods from published literature

Contaminants	Type of Plant	Contaminated Media	Spiked or Unspiked	Chamber Type	Flow Rate	Duration	Type of Sorbent/Media	Source
DCB, TCB[a] (Chlorinated Hydrocarbon)	*Phragmites australis* (wetland grass)	Hydroponic C14-OC solution	Spiked	Glass bell sealed at the base with a paraffin film	NL[b]	30 minutes twice a day; for 28 days	Scintillation Liquid	(San Miguel, Ravanel, and Raveton 2013)
Hg(II) (Metal)	*Arabidopsis thaliana*	1.5 ml of 25uM HgCl2 solution	Spiked	Closed-tube incubation	3 cm^3/sec= 180ml/min	10 minutes	Gold foil; Jerome 431 Mercury Vapor Analyzer	(Heaton et al. 1998)
Naphthalene (PAH)	Unsaturated zone soil with poplar trees	DNAPL 3.7m below ground surface; Naph. =500 µg/L	Unspiked	Stainless steel tray w/ open bottom flux chamber	2 L/min by SKC, Inc. PCXR8 personal sampling pump	Ran pump for 5 min. to create steady state, then ran for 3 days	Two XAD-2sorbent tubes (SKC, Inc.)	(Marr et al. 2006)
TCE (VOC)	Bald Cypress Tree	gravel/sand substrate with water level above surface; added 200-300 uL pure TCE	Spiked	Glass-carboy mesocosm	No pump	10 min. for a 30 min. time series	Roots sealed in serum vials	(Nietch, Morris, and Vroblesky 1999)
Se (Metal)	Cattail, baltic rush, saltgrass, tule, widgeon grass	water flow-through wetland cells	Spiked	6.6 mm thick Plexiglas	vacuum pump: 0.85m^3/hr = 14,166 mL/min	24 hr sampling duration; rates monitored for 2 years	charcoal filters	(Lin and Terry 2003)
Se (Metal)	Rabbitfoot grass seeds	Soil 510 mg Se added to soil; 170mg of Se as selenate	Spiked	6.6 mm thick Plexiglas	NL	38 days	charcoal filters	(Lin and Terry 2003)
TCE (VOC)	Carrots, spinach, tomatoes	140 µg/L; 560 µg/L soil		Vegetables grown in ceramic pots	NL	3.5-15 weeks	Liquid ethylene glycol monomethyl ether traps	(Orchard et al. 2000)
TCE (VOC)	Poplar	50 mg/L soil		Root and foliage separated by teflon tape; foliage chamber	0.8-1.1 L/min flow rate	1 week	Liquid ethylene glycol monomethyl ether traps	(Orchard et al. 2000)
As (Metal)	Pteris vittata (fern)	6540 mg As/kg dry weight soil; fronds accumulated 3830-11020 mg/kg DW	Unspiked	Fern STEM in 20L low-density polyethylene bottle	NL	2-7 days	none	(Sakakibara et al. 2010)

[a] DCB = Dichlorobenzene, TCB = Trichlorobenzene

b NL= Not Listed

Other categories of contaminants are present at the site, but are not the focus of the phytoremediation soil treatability study. The contamination concentrations in Area IV are extremely varied due to concentrated spills, building demolition, the wildfire, and other site disturbances. Table 2.2 shows the wide range of concentrations present in Sub-Area 5B. These values come from an expansive dataset developed to characterize the contamination in Area IV.

Table 2.2. Sub-Area 5B soil contaminant concentration ranges

Contaminant	Range in Sub-Area 5B
TPH EFH (C15-C20)[a]	0.41 - 22 ppm
PAHs	0.34 - 53000 ppb
PCBs	0.34 - 18000 ppb
Dioxins/Furans	0.00516 - 130000 ppt
Mercury	0.0028 - 23.6 ppm[b]

[a] TPH Extractable Fuel Hydrocarbons (EFH) equivalent carbon chain length of 15 to 20 carbon atoms (C15-C20)

[b] Parts per million (ppm), parts per billion (ppb), and parts per trillion (ppt)

Petroleum Hydrocarbons: Petroleum hydrocarbons (PHCs) is a term that describes a class of chemicals that originate from crude oil and is a mixture of hundreds of compounds that are primarily formed from carbon and hydrogen. Santa Susana Area IV was contaminated with PHCs through onsite use and disposal of petroleum based fuels (Department of Energy 2003). PHCs can cause nerve disorders, affect the blood and immune system, affect reproduction, and can cause cancer (ATSDR 2014).

Polyaromatic hydrocarbons: Polyaromatic hydrocarbons (PAHs) are a subset of PHCs that are of particular concern due to their stability and persistence in the environment. They are composed of two or more benzene rings fused together, hence the "polyaromatic" part of the name. Most PAHs have a high affinity for soil (not water) as indicated by high octanol-water partitioning coefficients (K_{ow}). For example pyrene, a four ringed PAH, has a log K_{ow} of 4.88 compared to a log K_{ow} of 2.18 for benzene. PAH contamination was introduced to Area IV through open burning of wastes, burning of rocket and vehicle fuels, and incomplete combustion of vegetation during the 2005 wildfire (Boeing 2005). Many PAHs are reasonably expected to be carcinogenic and suspected to cause birth defects (ATSDR 2014).

Polychlorinated biphenyls: PCBs are man-made chlorinated organic compounds. The structure of PCBs consists of two benzene rings attached by a single bond with a varied amount of chlorines attached to carbons in the benzene ring. Each PCB with a different arrangement and number of chlorines is referred to as a congener (Figure 2.2). PCBs are often known by their industrial trade names, the most common being Aroclor (EPA 2013b). PCBs are known as persistent organic pollutants (POPs) because of their high thermal and chemical stability due to their highly chlorinated aromatic structure Campanella et al. (2002). A high log K_{ow} (4.46 – 8.18) causes PCBs to accumulate in soils and sediments. In general, the more highly chlorinated the congener, the less water-soluble and volatile it is (Campanella, Bock, and Schröder 2002). Highly chlorinated PCBs are also harder to biodegrade. PCB congeners with 5 or more chlorine atoms must undergo

anaerobic reductive dechlorination to 3 or less chlorine atoms before they can be aerobically degraded (Aken, Correa, and Schnoor 2010). PCBs were used as coolants in transformers and electrical equipment in Area IV because of their insulating properties. Chronic exposure to PCBs can have serious neurological and immunological effects on children and they have been determined to be probably carcinogenic to humans by the EPA and International Agency for Research on Cancer (ATSDR 2014).

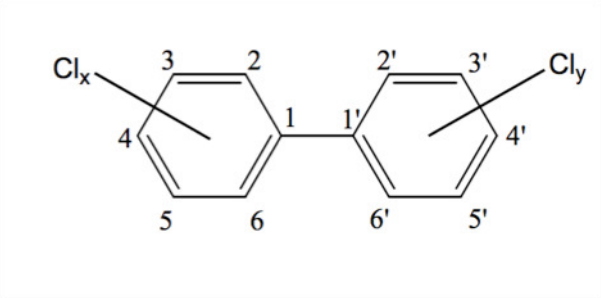


Figure 2.2. Molecular structure of PCBs

Chlorinated Dioxins/Furans: The term "dioxin" is often used to refer to polychlorinated dibenzo-p-dioxins (PCDDs), which have similar physical and chemical properties as PCBs. The dioxin molecule is a central part of PCDDs which are the compounds of primary concern. Compounds that contain furan such as polychlorinated dibenzofurans (PCDFs) are very closely related to PCDDs and are often grouped together in discussion because of their similar structure and chemical properties (EPA 2011). In this report the term "dioxins/furans" refers to both PCDDs and PCDFs. PCDD/Fs consist of a dioxin or furan center that links two benzene rings together that have 8 or less chlorine atoms bonded to the carbon atoms of the benzene rings (Figure 2.3). PCDD/Fs are even more hydrophobic than PCBs having log K_{ow} values from 7-10 which cause them to bind tightly to soil (Campanella, Bock, and Schröder 2002). Like PCBs, the compounds with different number and positioning of chlorines are referred to as congeners. They also follow the same trend that the more highly chlorinated the congener, the less water-soluble and volatile it is (Campanella, Bock, and Schröder 2002). They differ from PCBs in that they are formed through both natural and industrial combustion processes (Lemieux, Lutes, and Santoianni 2004; ATSDR 2014). The most toxic congener is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and all other dioxin toxicity is evaluated relative to this congener (ATSDR 2014). In several animal studies exposure to TCDD has been shown to cause liver and immune system damage and the World Health Organization (WHO) has determined that 2,3,7,8-TDD is a human carcinogen (ATSDR 2014).

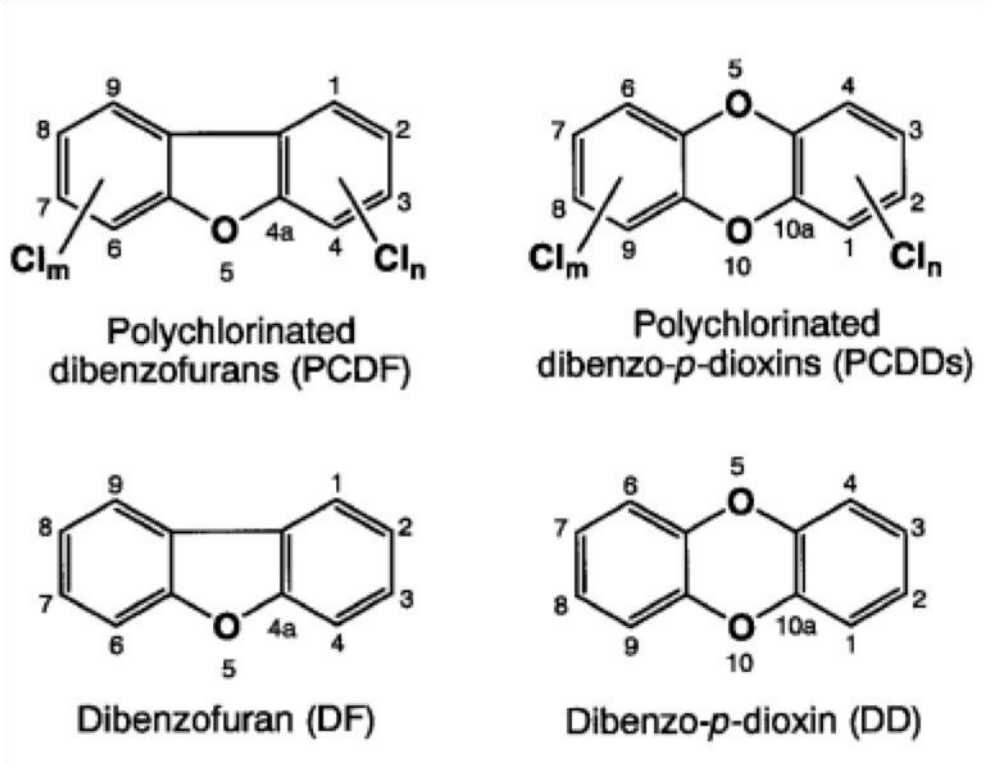


Figure 2.3. Generic chlorinated dioxins and furans

Metals: Metals are elements which are non-biodegradable and tend to accumulate in the environment and living organisms. Metals exist in either an elemental or oxidized state. For example, Hg can exist in the elemental form (Hg^0), the oxidized form (Hg^+), and the oxidized form (Hg^{2+}). Thus metals can be transported through the environment by dissolving into water or forming inorganic/organic compounds. Most metals do not volatilize readily, but mercury is the one exception and is often released into the atmosphere when mercury-containing coal is burned (EPA 2013a). Some of the metals that have contaminated Area IV are silver, cadmium, copper, mercury, lead, zinc, nickel, and chromium. Mercury is known to disrupt the nervous system, damage the brain, kidneys and lungs, and cause changes in vision and loss of memory in humans (ATSDR 2014). Other metals have similar toxic effects on humans. Metals are also extremely toxic to microorganisms in the environment and can also cause mutations, sickness, and death to plants at high concentrations (Giller, Witter, and Mcgrath 1998; Patra et al. 2004).

2.3.3. Treatment Goals

The Department of Toxic Substance Control created "Look-Up Tables" which provide target concentrations for acceptable levels of contamination for the site based on background contaminant concentrations. These tables separate major classes of chemicals (PCBs, PAHs, TPH, etc.) and give values for a multitude of compounds within those classes.

2.3.4. Site Vegetation

The vegetation in SSFL Area IV is made up of a mixture of native, naturalized, and invasive plant species. Recent site disturbances have changed many parts of the vegetative cover. Much of Area

IV was burned in a 2005 wildfire, and in 2010 the EPA cut back most of the vegetation to conduct radiological surveys of the entire area (HydroGeoLogic 2010). One benefit of phytoremediation could be the re-vegetation of Area IV with native or naturalized plants. With re-vegetation in mind, invasive species were not considered to be viable phytoremediation candidates. The Sandia report (2012) listed a collection of plants species known to remediate the COIs in other studies and a list of plant species thought to be analogous to them that might be growing in Area IV. A shortened version of that list is reproduced in Table 2.3.

Table 2.3. Known phytoremediators and site alternatives

Contaminant Group	Plants shown to Demonstrate Remediation Potential	Suggested Onsite Alternative
TPHs	Clover/Alfalfa (*Trifolium sp.*)	Small-headed Clover, Creek Clover, White-Tipped Clover, Tomcat Clover, White Clover
	Fescue (*Festuca sp.*)	Red Fescue, Small Fescue, Rat-Tail Fescue
	Ryegrass (*Lolium multiflorum*)	Giant Ryegrass, Beardless Wildrye
	Bermuda Grass (*Cynodon dactylon*)	Bermuda Grass (naturalized in wet areas)
PAHs	Clover/Alfalfa (*Trifolium sp.*)	Small-headed Clover, Creek Clover, White-Tipped Clover, Tomcat Clover, White Clover
	Fescue (*Festuca sp.*)	Red Fescue, Small Fescue, Rat-Tail Fescue
	Ryegrass (*Lolium multiflorum*)	Giant Ryegrass, Beardless Wildrye
	Bermuda Grass (*Cynodon dactylon*)	Bermuda Grass (naturalized in wet areas)
PCBs	Pumpkin/Zucchini (*C. pepo*)	Buffalo Gourd (native), Pumpkin/Zucchini Gourd (naturalized), Gourd
	Clover/Alfalfa (*Trifolium sp.*)	Small-headed Clover, Creek Clover, White-Tipped Clover, Tomcat Clover, White Clover
	Fescue (*Festuca sp.*)	Red Fescue, Small Fescue, Rat-Tail Fescue
	Ryegrass (*Lolium multiflorum*)	Giant Ryegrass, Beardless Wildrye
	Willows (*Salix spp.*)	Red Willow, Arroyo Willow
	Bermuda Grass (*Cynodon dactylon*)	Bermuda Grass (naturalized in wet areas)
Dioxins	Poplar (*Populus sp.*)	Black Cottonwood
Metals	Indian Mustard (*Brassica juncea*)	Black Mustard, Mediterranean Mustard
	Sunflower (*Helianthus annuus*)	Canyon Sunflower, Common Sunflower, California Sunflower, Slender Sunflower, Bush Sunflower
	Barley (*Hordeum vulgare*)	Little Barley

2.4. Phytoremediation Research on Plant Candidates

Previous phytoremediation studies have been conducted using some plants which may be considered analogous to plants growing at SSFL. Canadian Horseweed (*Conyza canadensis*) was identified as a Cd accumulator in laboratory growth experiments using Cd-spiked soil as well as in field experiments at a Cd-contaminated site (Wei et al. 2009). Another study showed that Slender Wild Oat (*Avena barbata*) enhanced the biodegradation rates of phenanthrene in the rhizosphere and also increased the number of bacterial phenanthrene degraders in the soil (Miya and Firestone 2000). A subsequent study by the same authors showed an increase in phenanthrene degradation for soil amended with Slender Wild Oat root exudates and debris (Miya and Firestone 2001). Soft Chess (Bromus hordeaceus) was reported to accumulate a fair

amount of Ni (1467 mg/kg in the aerial parts) in a screening study of plants growing in an old mining area of Braganca, Portugal (Freitas, Prasad, and Pratas 2004). Another screening study of 32 different plant species showed that Summer Mustard (*Hirschfeldia incana*) had the best characteristics to phytoextract Cu from the soil (Poschenrieder et al. 2001). The Summer Mustard exhibited high Cu tolerance and high root to shoot transfer of Cu within the plant. In a 40-day pot experiment, Summer Mustard extracted large amounts of Zn and Pb from soil taken from a site historically contaminated by industrial activity (Gisbert et al. 2006). Summer Mustard also showed a high uptake of As from the alluvial flats of the Turia River in Valencia, Spain (Gisbert et al. 2008). Wild Oat (*Avena fatua*) accumulated more Pb than several other plant species and accumulated Pb mostly in the roots (Wu, Chen, and Tang 2005). Purple Needlegrass (*Nassella pulchra*) was used in combination with other native grasses (*Bromus carinatus*, *Elymus glaucus*, *Festuca ruba*, *Hordeum californicum*, *Leymus triticoides*) to reduce concentrations of petroleum hydrocarbons by 30% in two years (Siciliano et al. 2003). The Sierra Streams Institute also found that Purple Needlegrass accumulated five times as much lead as other non-accumulating species at two abandoned mine sites in Nevada City (Institute 2013). A study done with Mule Fat (*Baccharis salicifolia*) showed that copper is sequestered into the roots of the plant but not into the leaves (Cordale Johnson, John Thomlinson 2009).

2.4.1. Plant Characteristics Ideal for Phytoremediation

A wide variety of plant species have been identified as phytoremediators. From large trees to small grasses, many different plants exhibit certain characteristics that make them candidates for phytoremediation. The following are the primary phytoremediation characteristics (Anderson, Guthrie, and Walton 1993; Salt, Smith, and Raskin 1998; Pilon-Smits 2005; Memon and Schröder 2009; Ali, Khan, and Sajad 2013; Cook and Hesterberg 2013):

1. Rapid growth
2. Tolerance to toxic contaminants
3. High accumulation of contaminants in the roots
4. Efficient translocation of contaminants from roots to above ground plant tissue
5. Ability to produce large amounts of biomass
6. Dense/expansive root system
7. High production of degrading enzymes
8. Tolerance to environmental conditions
9. Resistance to pathogens and pests
10. Repulsive to herbivores
11. Easy to cultivate and harvest

The first seven characteristics listed are important for handling the contaminant itself, whereas the last four characteristics are important for the practical implementation of phytoremediation at a contaminated site. The accumulation potential of plants can be evaluated using the bioconcentration factor (BF) - the ratio of contaminant concentrations in the plant to that in the soil, and the translocation factor (TF) – the ratio of contaminant concentrations in the shoots to that in the roots (Marchiol et al. 2013; Baker and Brooks 1989). The BF indicates the plant's ability to extract contaminants from the soil and the TF indicates the plant's ability to transport the contaminants from the roots to the shoots of the plant.

2.5. Contaminant Bioavailability

The bioavailability of contaminants can limit the effectiveness of phytoremediation. Bioavailability is a measure of how accessible contaminants are to biological organisms. Plants and microorganisms must be able to access/contact the pollutant in order to start the biodegradation process. Metals, such as Pb, often form complexes with organic matter or precipitate with carbon, phosphorous, and hydroxide which limit Pb's availability for plant uptake (Blaylock et al. 1997). Hg at mining sites can also be largely unavailable because it is in the form of HgS (Wang et al. 2012). PCBs and other organic compounds with high octanol-water partitioning coefficients are often bound tightly to soil which prevents them from entering the soil-water phase where contaminant degrading microorganisms may reside (Providenti, Lee, and Trevors 1993; Shen et al. 2009).

Increased contaminant bioavailability allows plants and microorganisms to be more effective at remediation. Different factors can increase the bioavailability of compounds in soil. Surfactants, or chelating agents, can increase the bioavailability of organics or metals by forming a water-soluble compound or complex that can be absorbed into plant tissue or accessed by bacteria (Blaylock et al. 1997; Shen et al. 2009). Plants themselves can release compounds that increase contaminant water solubility and improve phytoextraction (Campanella, Bock, and Schröder 2002). Other factors such as pH and temperature can also increase or decrease bioavailability depending on the type of contaminant (Blaylock et al. 1997; Wang et al. 2012).

2.6. Chelating Agents and Surfactants

Chelating agents are chemical compounds that form a metal ion complex which can make the metal more mobile (Flora and Pachauri 2010). Ethylenediaminetetraacetic acid (EDTA) is a widely used synthetic chelating agent that has been shown to increase the uptake of metals into plants (Salt, Smith, and Raskin 1998). The Indian mustard plant (*Brassica juncea*) is well known for its ability to hyperaccumulate lead from soils amended with EDTA. Not only does EDTA increase the uptake of lead into *B. juncea*, but it also increases the translocation of lead from the roots into the shoots of the plant (Blaylock et al., 1997; Jianwei W. Huang et al., 1997). Ethylenebis[oxyethylenetrinitrilo]tetraacetic acid (EGTA) and (2-Hydroxyethyl) ethylenediaminetriacetic acid (HEDTA) have also been shown to increase the bioavailability of cadmium and lead for enhanced accumulation in *B. juncea* and *Zea mays* plants respectively (J. W. Huang and Cunningham 1996; Blaylock et al. 1997). The effectiveness of these chelating agents varies depending on the contaminant, soil characteristics, and the plant species.

Surfactants are chemicals that can decrease the sorption of contaminants to soils and increase the bioavailability of the contaminant. Cyclodextrins, which are non-toxic, biodegradable surfactants used in the food industry, have been shown to enhance the availability of PCBs in soil to improve uptake into plants (Fava, Di Gioia, and Marchetti 1998; Shen et al. 2009). Biosurfactants, such as glycolipids or rhamnolipids, can be used to increase the solubility of organic contaminants and are an appealing alternative to synthetic surfactants because of lower toxicity and enhanced biodegradation (Providenti, Lee, and Trevors 1993).

Harvesting plants to remove contaminants becomes more efficient and economically feasible when using chelating agents and surfactants to increase the rate of phytoextraction and improve

translocation within plants. However, care must be taken when enhancing the bioavailability of contaminants using chelates/surfactants because the contaminants will also be transported by groundwater or rain more easily.

2.7. Expected Effectiveness of Phytoremediation for COIs

***Petroleum Hydrocarbons**:* Based on published data, phytoremediation of PHCs could be effective. One study showed over 50% degradation of approximately 7000 ppm of TPH in one year (Phillips et al. 2009). Another study showed over 63% degradation of 5000 ppm of TPH in only 127 days (Peng et al., 2009). Slower rates have been reported by Banks et al. (2003), with 50% reduction of 3000 ppm of TPH observed in 870 days. Based on these studies, the time to remediate 5000 ppm of TPH down to the SSFL background level of 5.7 ppm could take between 1.3 and 23 years (assuming first order kinetics).

Polyaromatic hydrocarbons: Studies have shown remediation of PAHs through rhizosphere stimulation and even some phytoextraction into the tissue of several different plant species, although phytoextraction was limited (Gao and Zhu 2004; Hall, Soole, and Bentham 2011). For instance, a study by Gao and Zhu (2004) showed that phytoextraction alone contributed to only 0.01% (phenanthrene) and 0.24% (pyrene) of the total PAH reduction by plants. The rates of removal vary depending on the specific PAH, the plant species, and concentration of contaminant. The phytoremediation rates for PAHs can be very rapid as shown in one study that removed 67.5% of pyrene from soil in 28 days (Liste and Alexander 2000). Based on the published studies, phytoremediation of PAHs could reduce soil PAH concentrations at SSFL from 50 ppm (typical value at SSFL) to 0.00447 ppm (background level) on a time scale of 1.5 to 2.7 years. This estimate is based on first order rate constants for various PAHs calculated in a study by Robinson et al. (2003). However, slower degradation rates (9.1% reduction in a year) have been observed with weathered soil that could indicate a longer remediation time of around 98 years at SSFL based on first order kinetics (Parrish et al., 2004). Clearly phytoremediation rates of PAHs are site specific, and studies would need to be conducted specifically with site soils to provide a narrower estimate of phytoremediation rates at the site.

Polychlorinated biphenyls (PCBs): Both phytoextraction and rhizoremediation have been shown to be operative mechanisms of remediation of PCBs in soil (Aken et al., 2010). Alfalfa and other plant species have been shown by several studies to extract PCBs from soil (Ficko et al., 2010; Greenwood et al., 2011; Liu & Schnoor, 2008; Xu et al., 2010; Teng et al., 2010; Zeeb et al., 2006). However, PCB degradation within the plant does not appear to be the primary mechanism of phytoremediation. Several studies have suggested that rhizoremediation was the primary mechanism of PCB degradation at specific field sites, and in these studies stimulated enzyme activity and increased microbial populations in the root zone supported this hypothesis (Chekol et al., 2004; Y. Li et al., 2013; Xu et al., 2010; and Teng et al., 2010). Field and lab studies reported varying phytoremediation rates for PCBs, with rates ranging from 8.1% removal in 180 days to 77% removal in 122 days. The type of PCB congener, the plant species, and the PCB concentration impact the rates of removal. Given the observed rates of removal cited in the literature, phytoremediation for PCBs at a soil concentration of 10 ppm could be effective on a time scale of 2 to 50 years assuming first order kinetics and a final concentration of 0.017ppm (look-up table).

Chlorinated Dioxins/Furans*:* Chlorinated Dioxins are similar to PCBs in their chemical structure and properties but limited research has been done on dioxin phytoremediation. Studies done with zucchini (*Cucurbita pepo*) have shown that this species can efficiently phytoextract dioxins from soil (Campanella & Paul, 2000; Huelster et al., 1994). Plants can stimulate rhizosphere dioxin degradation in the same way they stimulate PCB rhizosphere degradation (Campanella et al., 2002). The degradation of dioxins through phytoremediation has not been adequately studied to estimate a remediation time scale.

Metals: Metal phytoremediation can be pursued as an active bioremediation strategy. There is an abundance of studies that show uptake of heavy metals by plants, and it is estimated that over 500 plant taxa are hyperaccumulators of metals (Ent et al. 2013). Phytoremediation can be effective given enough time, proper harvesting, and adequate planting density/speciation, and any necessary amendments such as chelating agents. However, the specific time for complete remediation can be highly variable depending on these factors.

3.0. Methodology

3.1. Phase I: Field Screening

The purpose of Phase I of this study was to identify a list of plant species as candidates for phytoremediation for further testing. Uptake of COIs by plants growing in the field at SSFL was measured as an indicator of phytoremediation potential. Once the best species were identified they were then grown in the Phase II greenhouse experiments.

3.1.1. Plant Selection and Tagging for Phase I Field Sampling

On a site visit in May 2013, various plants species of trees, shrubs, forbs, and grasses were observed growing in areas with known contamination based on extensive prior soil sampling. These species, along with some others that have historically grown on the Site, were combined to form a list of potential phytoremediation candidates (Table 3.1). An 'A' in a contaminant column indicates that the plant is an analog of a species listed as a potential phytoremediator in the Sandia Soil Treatability Study. Plant species were given priority if they fulfilled the following criteria: (1) native to SSFL, (2) candidate for restoring (revegetating) Area IV, (3) abundant enough in Area IV to allow adequate sampling, (4) high rate of growth, (5) known to uptake contaminants.

The list of plant species in Table 3.1 was further narrowed down to nine species to be sampled and analyzed for COIs. Three of each plant species growing in contaminated soil and one of each species growing in uncontaminated soil were identified for harvesting and analysis. The contaminated areas were selected based on the following target contaminant concentrations: TPH 500 ppm, PAH 1000 ppb, PCBs 1000 ppb, chlorinated dioxins/furans 100 ppt, and mercury, silver, lead, and zinc at levels exceeding the Look-Up Table values. The control locations were based on previous sampling data points with COI concentrations less than or equal to twice the Look-Up Table value. Using the data provided by CDM Smith, sampling locations were screened to identify concentrations of COIs measured at previous sample locations that fit both the contaminated criteria and control criteria. Sub-Areas 5A, 5B, and 5C, were selected as areas in which to search for plant species in contaminated soil based on prior soil analyses. For the control plants, the locations of suitable and unsuitable previous sampling points were mapped using the GIS software ArcMap 10.1. Figure 3.1 shows the locations of suitable and unsuitable control sample points. Control points located in BZ-NW were chosen for the control area for plant specimens.

Table 3.1. Potential phytoremediation candidates at SSFL

2012 Jepson Manual Scientific Name[a]	Common Name	Origin	Habit	Duration[b]	Restoration[c]	Abundance[d]	Annual Growth	Dioxin	PCB	PAH	TPH	Metals	Hg[f]
Sambucus nigra	Blue Elderberry	Native	Shrub/Tree	Perennial	Yes	Low	Medium						
Malosma laurina	Laurel Sumac	Native	Shrub	Perennial	Yes	Medium	Medium						
Artemisia californica	California Sagebrush	Native	Shrub	Perennial	Yes	Medium	Low					A	
Baccharis salicifolia	Mule-Fat	Native	Shrub	Perennial	Yes	High	Medium					A	
Deinandra minthornii	Santa Susanna Tarweed	Native	Shrub	Perennial	Yes	Medium	Low					A	
Ericameria palmeri	Palmer Goldenbush	Native	Shrub	Perennial	Yes	High	Medium					A	
Heterotheca grandiflora	Telegraphweed	Native	Forb	Annual	NO	Medium	High					A	
Hirschfeldia incana	Summer Mustard	Alien	Forb	Perennial	NO	High	High					A	A
Acmispon americanus	Spanish Lotus	Native	Forb	Annual	Yes	Low	Medium		A	A	A		
Acmispon glaber	Common Deerweed	Native	Shrub	Perennial	Yes	Medium	Medium		A	A	A		
Eriogonum fasciculatum	California Buckwheat	Native	Shrub	Perennial	Yes	High	Medium						
Salix lasiolepis	Arroyo Willow	Native	Tree/Shrub	Perennial	Yes	Low	Medium	A[e]	A				A
Avena fatua	Wild Oat	Alien	Grass	Annual	NO	High	High					A	
Stipa cernua	Nodding Needlegrass	Native	Grass	Perennial	Yes	Medium	Low						
Stipa pulchra	Purple Needlegrass	Native	Grass	Perennial	Yes	Low	Low						
Rhus ovata	Sugar Bush	Native	Shrub	Perennial	Yes	Low	Medium						
Asclepias fascicularis	Narrowleaf Milkweed	Native	Forb	Perennial	Yes	Low	Medium						
Baccharis pilularis	Coyotebrush	Native	Shrub	Perennial	Yes	Medium	Medium					A	
Brickellia californica	California Bricklebush	Native	Shrub	Perennial	Yes	Low	Medium					A	
Corethrogyne filaginifolia	Common Sandaster	Native	Forb	Perennial	Yes	Medium	Medium					A	
Erigeron canadensis	Canadian Horseweed	Native	Forb	Annual	NO	Medium	High					A	

2012 Jepson Manual Scientific Name[a]	Common Name	Origin	Habit	Duration[b]	Restoration[c]	Abundance[d]	Annual Growth	Dioxin	PCB	PAH	TPH	Metals	Hg[f]
Pseudognaphalium californicum	California Cudweed	Native	Forb	Perennial	Yes	Medium	High					A	
Eriodictyon crassifolium	Thickleaf Yerba Santa	Native	Shrub	Perennial	Yes	High	Medium						
Ribes malvaceum	Chaparral Current	Native	Shrub	Perennial	Yes	Low	Medium						
Salvia leucophylla	Purple Sage	Native	Shrub	Perennial	Yes	Medium	Medium						
Salvia mellifera	Black Sage	Native	Shrub	Perennial	Yes	Medium	Medium						
Rhamnus ilicifolia	Hollyleaf Redberry	Native	Shrub	Perennial	Yes	Low	Medium						
Prunus ilicifolia	Hollyleaf Cherry	Native	Tree/Shrub	Perennial	Yes	Medium	Medium						
Avena barbata	Slender Wild Oat	Alien	Grass	Annual	NO	High	High					A	
Bromus diandrus	Ripgut Brome	Alien	Grass	Annual	NO	High	High			A		A	
Bromus hordeaceus	Soft Chess	Alien	Grass	Annual	NO	High	High			A		A	
Bromus madritensis	Red Brome	Alien	Grass	Annual	NO	High	High			A		A	

[a] Scientific name from the 2012 Jepson Manual: Vascular Plants of California

[b] Duration indicates the life expectance of the plant

[c] These plants could be used to restore the vegetation of Area IV

[d] The plant abundance in Area IV as observed during May 30th, 2013 site visit

[e] The 'A' indicates that the plant is analogous to a species show to uptake this contaminant and identified by the Sandia Study

[f] Hg is the abbreviation for mercury

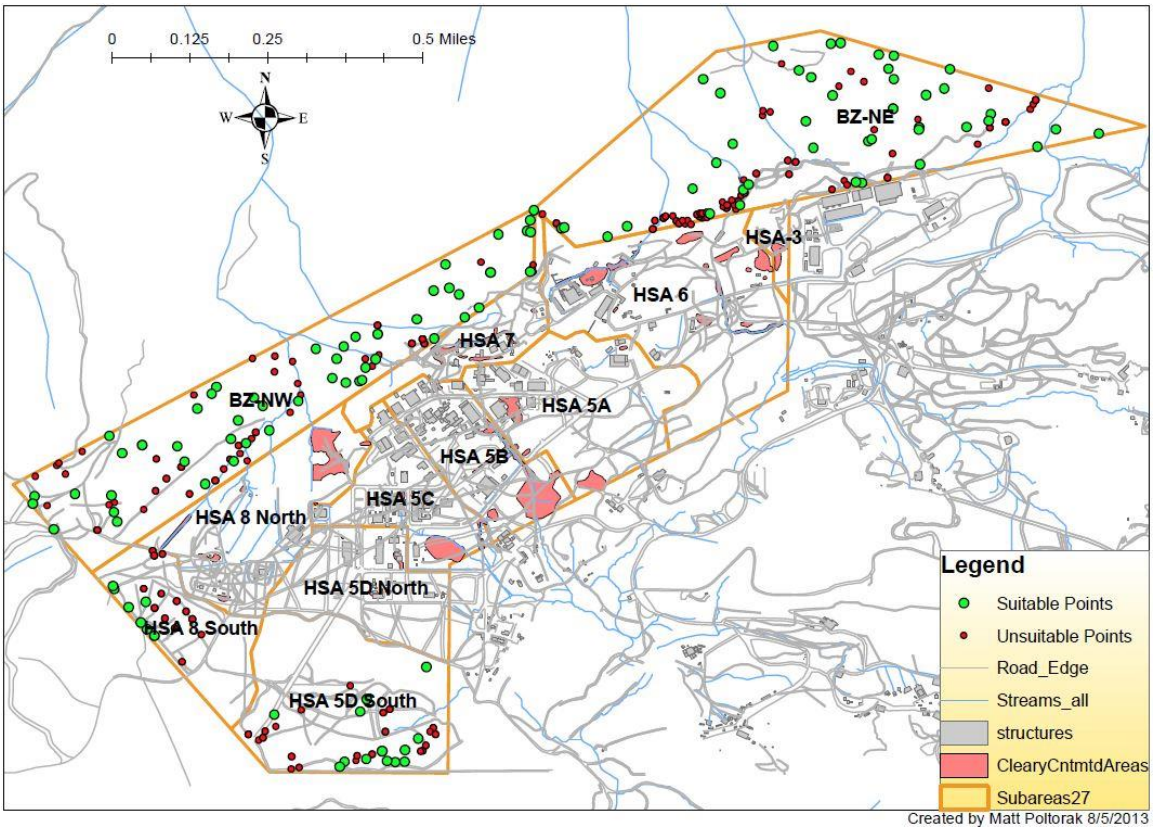


Figure 3.1. Sampling points for plant control sampling in SSFL Area IV

The site-selection criteria were used during a site visit on August 8, 2013 to find a mix of shrubs, forbs, and grasses from Table 3.1 that were suitable for sampling. Nine plant species were selected for screening:

- Purple Needlegrass (*Stipa cernua*),
- Blue Elderberry (*Sambucus nigra*),
- Laurel Sumac (*Malosma laurina*),
- Mule Fat (*Baccharis salicifolia*),
- Palmer's Goldenbush (*Ericameria palmeri*),
- Summer Mustard (*Hirschfeldia incana*),
- Narrowleaf Milkweed (*Asclepias fascicularis*),
- Coyote Brush (*Baccharis pilularis*), and
- Thickleaf Yerba Santa (*Eriodictyon crassifolium*).

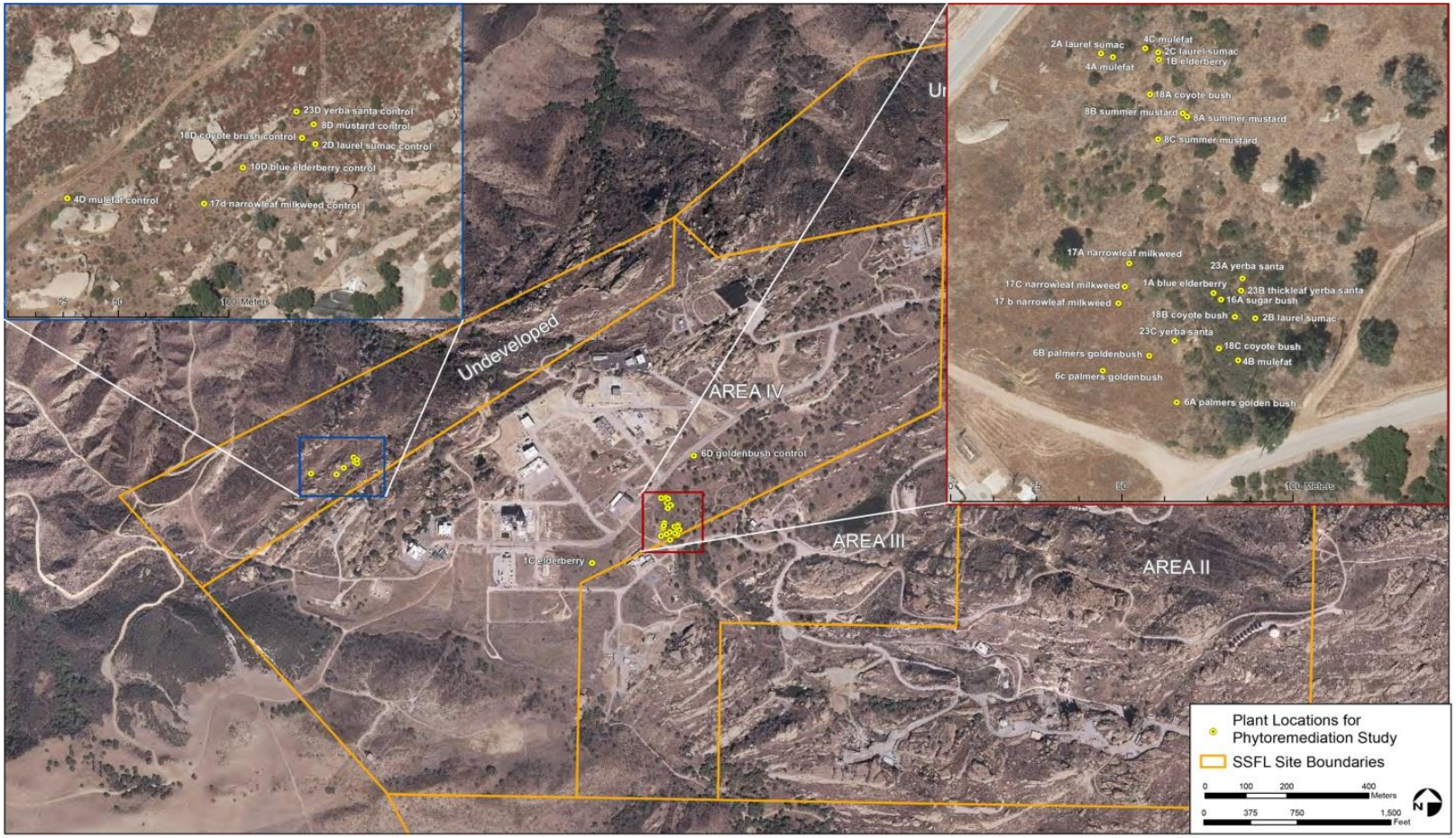
Plants found to be growing in Area IV were labeled using flagging tape and the GPS coordinates were recorded. Plant specimens were labeled with a number (1 through 32) based on their position in Table 3.1, and a letter (A-D) for each separate specimen within the sample species. Control specimens were labeled with the letter D. For example, the control specimen for Blue Elderberry was labeled Specimen 1D. Green plants of moderate size were tagged while dried plants were not tagged because some contaminants are likely to have escaped during the drying process. If a tagged plant was no longer suitable for sampling at the actual date of sampling, then a new specimen was found and labeled by adding an additional letter to the tag. For example, Narrowleaf Milkweed 17A was changed to 17AA. Figure 3.2 shows the locations of the plants tagged on August 13, 2013.

3.1.2. Plant Tissue and Root-Zone Soil Sampling

All of the tagged plant specimens and their root-zone soil were sampled and analyzed for COIs. Due to the different growing seasons of the candidate plants, the soil and plant tissue were collected on two different sampling events so that tissue samples were collected during periods of plant growth when contaminant uptake was expected to be the greatest. The roots and above-ground mass (AGM), comprised of stem and leaves, were analyzed separately.

The whole plant, including roots, was removed from the soil using a stainless steel shovel decontaminated between composite samples. The soil attached to the roots of the plant was brushed into a stainless steel collection bowl. Soil from around the root zone was added to the collection bowl to meet the required mass for the soil sample listed in Table 3.2. The soil taken from the root zones of the plants was homogenized using the cone and quarter method in the onsite DOE workstation.

After the soil samples were collected, the AGM portion of the plant was separated from the roots with stainless steel plant clippers that were decontaminated between plant samples. Both parts of the plant were placed into separate Ziploc bags and transported to the onsite DOE lab. Once in the lab, any soil attached to the foliage of the plant was rinsed off using deionized (DI) water. The DI water was transported to the site for use in the field sampling events. The cleaned plant was air dried by resting on a paper towel for 1 hour inside an onsite DOE workstation. A composite tissue sample was prepared by cutting stems and leaves from different areas of the plant to meet the mass requirements (Table 3.2). The aliquots were weighed using a balance to ensure sufficient mass was collected, placed in a wide-mouth, 500-mL glass container, and stored in a cooler at <4°C. A portion of the collected tissue was set aside for Lancaster laboratories to perform the dioxin/furan, total organic carbon (TOC), moisture, and total nitrogen analysis. The rest of the analytes were analyzed by EMAX laboratories using the remaining plant tissue.



Vegetation Monitoring Sites for Cal Poly

Figure 3.2. Location of tagged plants in Area IV on August 13, 2013

Table 3.2. Required sample mass for analytical methods

			Laboratory			
			Day 0	Day 85[b]	Day 211	
Contaminant	**Soil[a]** (g)	**Plant Tissue** (g)	**Soil/Plants**	**Soil**	**Soil**	**Plants**
PCB	30	5	EMAX	EMAX	Lancaster	EMAX
PAH	30	5	EMAX	EMAX	Lancaster	EMAX
TPH	15	5	EMAX	EMAX	Lancaster	N/A
Metals	5	3	EMAX	EMAX	Lancaster	EMAX
Mercury	3	3	EMAX	EMAX	Lancaster	EMAX
Dioxin	10	5	Lancaster	Lancaster	Lancaster	EMAX
Moisture	10	N/A	Lancaster	Lancaster	Lancaster	N/A
Nitrogen	75	N/A	Lancaster	Lancaster	Lancaster	N/A
Organic Carbon	50	N/A	Lancaster	Lancaster	Lancaster	N/A
Total	**228**	**26**				

[a] These masses are based on an estimated soil moisture of 12%

[b] Plant tissue was not sampled on Day 85

Once in the DOE laboratory, the roots of the plant were soaked in a bucket of DI water and carefully scrubbed with a vegetable brush to separate the roots from any remaining soil. The brush was decontaminated between samples. The cleaned roots were air dried by resting on a paper towel in a shady spot for 1 hour inside the DOE laboratory. A composite sample was prepared by cutting roots from different areas of the plant to meet the mass requirements (Table 3.2). The aliquots were weighed using a balance to ensure sufficient mass was collected, placed in a wide mouth 500-ml glass container, and stored in a cooler at <4 °C. A portion of the collected tissue was set aside for Lancaster laboratories to perform the dioxin/furan, total organic carbon (TOC), moisture, and total nitrogen analysis. The rest of the analytes were analyzed by EMAX laboratories using the remaining plant tissue. The analytical methods used by the laboratories are listed in Table 3.3.

The minimum amount of plant tissue required was 26 grams, but 100 grams were collected as a safety factor. When there was insufficient plant tissue to obtain the required 100 grams, two or more plants in close proximity were collected and homogenized by the contracted laboratories according to their standard procedures.

Table 3.3. EPA methods used to analyze soil and plant samples

Contaminant	Soil	Plant Tissue
PCB	EPA Method 1613B	EPA Method 1668C
Dioxin	EPA Method 1613B	EPA Method 1613B
PAH	EPA Method 8270C/D SIM	Gas Chromatograph/ High Resolution Mass Spectroscopy (GC/HRMS): EPA Method
TPH	EPA Method 8015B/C/D	Gas Chromatograph/Flame Ionization Detector (GC/FID): EPA Method
Mercury	CVAAS: cold vapor atomic absorption spectroscopy (EPA Method 7471A)	Cold Vapor Atomic Absorption (CVAA): EPA Method
Metals	EPA Method 6010C/6020A/7471A	Inductively Coupled Plasma/High Resolution Mass Spectroscopy (ICP/HRMS): EPA Method

3.2. Phase II: Microcosm Experiment Methods

3.2.1. Soil Collection for Microcosms

Sixty-five gallons of contaminated soil from Sub-Area 5B was collected for use in the phytoremediation microcosm experiments. The top few inches of soil and debris was scraped away using a stainless steel shovel and soil was gathered from a maximum depth of two feet. The soil was mixed in a large container in the field using stainless steel shovels (Figure 3.3). A gallon of DI water was added during mixing to reduce dust. The soil was then transported to Cal Poly in Teflon-lined 5-gallon buckets. Soil was sieved through a #4 sieve (4.750 mm openings) to remove debris or large rocks.

Figure 3.3. Collection of bulk soil for microcosm experiment

3.2.2. Microcosm Soil Homogenization

After sieving, additional homogenization was performed using stainless steel shovels. The soil was separated into four piles, then piles opposite to each other were mixed until one large pile was formed. This process was repeated for several hours to complete the homogenization process (Figure 3.4).

Figure 3.4. Bulk homogenized soil

Analytical M After the soil samples were collected, the AGM portion of the plant was separated from the roots with stainless steel plant clippers that were decontaminated between plant samples. Both parts of the plant were placed into separate Ziploc bags and transported to the onsite DOE lab. Once in the lab, any soil attached to the foliage of the plant was rinsed off using deionized (DI) water. The DI water was transported to the site for use in the field sampling events. The cleaned plant was air dried by resting on a paper towel for 1 hour inside an onsite DOE workstation. A composite tissue sample was prepared by cutting stems and leaves from different areas of the plant to meet the mass requirements (Table 3.2). The aliquots were weighed using a balance to ensure sufficient mass was collected, placed in a wide-mouth, 500-mL glass container, and stored in a cooler at <4°C. A portion of the collected tissue was set aside for Lancaster laboratories to perform the dioxin/furan, total organic carbon (TOC), moisture, and total nitrogen analysis. The rest of the analytes were analyzed by EMAX laboratories using the remaining plant tissue.

3.2.3. Analytical Methods

The analytical methods used to analyze the microcosm soil and plant tissue are identical to those used for Phase I field sampling (Table 3.3). For volatilization sampling a modified EPA Standard Method #3550 was used. Section 3.2.8 describes this method in greater detail.

3.2.4. Decontamination of Sampling Equipment

All tools that were used during the preparation or sampling in the field or for the laboratory microcosms were decontaminated. An Alconox® detergent solution was prepared in a plastic bucket. The tools were scrubbed thoroughly in the bucket with a sponge soaked in Alconox® solution. The washed tools were then rinsed with DI water and dried with a paper towel. The tools were decontaminated before contact with the microcosm, between duplicate sampling in a microcosm, between the sampling of two separate microcosms and before storing the tools.

3.2.5. Microcosm Construction

One-gallon glass jars served as the microcosm containers. Since there was no drainage port, glass marbles were placed at the bottom of each jar and overlaid with a fiberglass mesh to support the soil mass above. This created airspace for water to seep into which allowed the soil to be watered and stay aerobic by keeping the soil separate from standing water. A hollow glass tube was inserted from the top of the soil down to the fiberglass mesh to provide passive airflow to prevent anaerobic conditions. All of the glass was washed with an Alconox® solution and then with 10% nitric acid before contacting the soil from the site. A planted microcosm is diagramed in Figure 3.5 and an actual microcosm is shown in Figure 3.6.

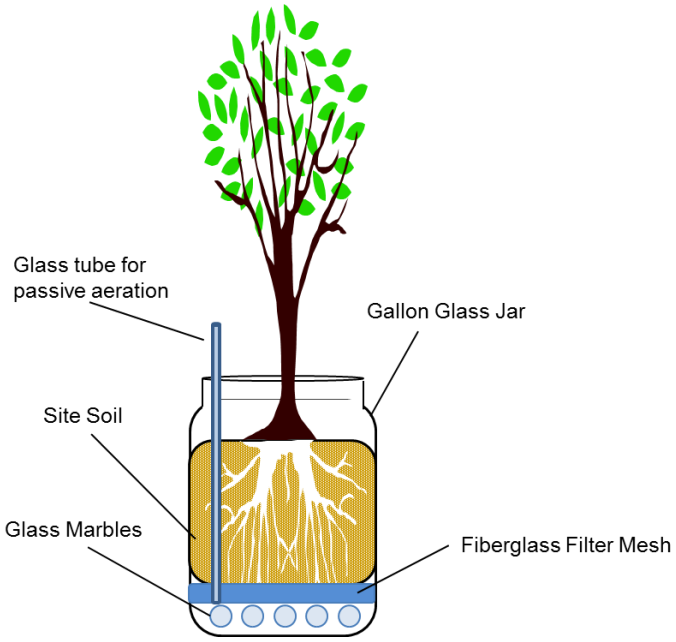


Figure 3.5. Planted microcosm

Figure 3.6. Microcosm profile view

3.2.6. Microcosm Conditions

The microcosms used in the controlled laboratory growth experiments were designed to mimic the following Area IV field conditions:

1. Temperature
2. Humidity
3. Sunlight
4. Soil moisture
5. Soil type

A total of 40 microcosms were prepared. Three plant species exhibiting the greatest contaminant uptake in the field based on the Phase I analytical results were selected for growth in laboratory microcosms to determine phytoremediation rates and mechanisms. Coyote Brush (*Baccharis pilularis*), Mule Fat (*Baccharis salicifolia*), and Purple Needlegrass (*Nassella pulchra*) species were chosen and grown with 5 replicates for each microcosm treatment (Table 3.4). One microcosm set (5 replicates, plus 5 extra for microcosms containing plants) of each of the three species was planted in the microcosm soil with no amendment additions or sterilization.

Table 3.4. Overview of the microcosm laboratory experiment

Sample Identification	Plant tissue sampled?	Chelating additive?	TRF[a] analysis?	5 extra microcosms?
Coyote Brush	Yes	No	No	Yes
Mule Fat	Yes	No	No	Yes
Purple Needlegrass	Yes	No	Yes	Yes
Coyote Brush w/fertilizer	Yes	No	No	Yes
Coyote Brush w/chelation	Yes	Yes	No	Yes
Purple Needlegrass Sterilized Planted Control	Yes	No	Yes	Yes
Unplanted Control	No	No	Yes	No
Sterilized Unplanted Control	No	No	Yes	No

[a] TRF stands for terminal restriction fragment analysis, a method for determining difference in microbial populations

One set of Coyote Brush planted microcosms was fertilized with Miracle-Gro® to test the effect of added fertilizer and determine if adequate nutrients were available in the field. The active constituents of Miracle-Gro® are nitrogen 24%, Phosphate (P_2O_5) 8%, soluble potash (K_2O) 16%, boron 2%, soluble copper 0.07%, chelated iron 0.15%, chelated manganese 0.05%, molybdenum 0.0005%, and soluble zinc 0.06%. Miracle-Gro® fertilizer was dissolved in DI water and added in 5 doses for a final concentration of 0.237 g fertilizer/kg of soil.

The chelating agent EDTA was added to another set of Coyote Brush microcosms to test if it aided in the phytoremediation process by increasing the bioavailability of metals in the microcosm soil. Hydrated ethylenediaminetetraacetic acid (EDTA) with a drying loss of 9.4% was dissolved in DI water and added in 4 doses for a final concentration of 6 mmol EDTA/kg of soil. A set of unplanted microcosms was operated with soil from the site to observe biodegradation rates associated with soil microbes only, without any phytoremediation mechanisms. The results from the unplanted soil control were compared with those from the planted microcosms to determine biodegradation rates in soil independently from any plant effects.

Sterilized controls were run with soil sterilized by gamma irradiation using cobalt-60 at the Sterigenics sterilization facility in Gilroy, CA. The soil was irradiated until it was exposed to at least 25 kGy (and as high as 62 kGy). Purple Needlegrass was selected for growth in one sterilized set because it exhibited the greatest rhizostimulation potential based on the literature review. Another sterilized microcosm set was unplanted and covered with a lid to prevent the escape of contaminants. The two purposes of these sterilized controls were (1) to help elucidate the mechanisms of phytoremediation by comparing biodegradation rates in planted microcosms with and without active soil microbes, thus quantifying the role of the soil microbes in contaminant degradation, and

(2) to control for any abiotic contaminant losses from unplanted sterile soil using the same experimental set-up.

The combined results of the sterile unplanted controls, sterile planted controls and planted microcosms with active soil microbes were used to test for stimulation of microbial biodegradation by the plants. By simply comparing planted to unplanted (both with active microbes), any additional contaminant loss in the soil with plants could either be from the plant stimulating the soil microbes or the plant itself extracting or degrading the contaminants. Further, by comparing results from plants in sterile soil to plants in soil with active microbes, the role of the plants could be identified.

The effect of plants on the microbial community of the root zone was characterized by comparing terminal restriction fragment (TRF) analyses of DNA extracted from soils with and without plants. The TRF method uses restriction enzymes to produce different lengths of DNA associated with each bacterial/fungal species. The differences in patterns of these DNA strands were used to compare the microbial community structure of planted and unplanted soil. This was used to provide possible evidence for phytostimulation of root-zone biodegradation.

3.2.7. Midpoint Microcosm Sampling (85 days)

The midpoint microcosm soil-sampling event was conducted on June 18 and 19, 2014, about 85 days after planting. Table 3.5 lists all of the microcosm treatments that were sampled. The following procedure was used to sample each type of microcosm:

1. The soil samples were taken from an area between the three plants and from a depth of 0.0 to 0.5 ft with as little disturbance of the plants as possible. For purple needlegrass it was impossible to avoid disturbing the roots during the sampling as the grass covered the entire soil surface.
2. Two stainless steel spoons were used to scoop soil from the microcosms into the sampling jars.
3. At least 120 g of soil was collected in an 8-oz jar for Lancaster Lab, and at least 40 g of soil was collected in a 4-oz jar for EMAX Lab for each microcosm jar.
4. The stainless steel spoons were decontaminated between microcosm types.
5. Steps 1-4 were repeated for each type of microcosm.
6. Duplicate soil samples were taken from two jars for QA/QC.

For transport, the 4oz and 8oz jars were placed into padded bags and sealed in a quart sized Ziploc bag. An equipment blank was prepared for the sampling event by running DI water over the stainless steel spoons and into the sample jar. The samples were shipped on ice with a holding time of two days maximum.

Table 3.5. List of sampled microcosms at 85 days

Sampled Microcosm Types		Number of jars sampled	Date Sampled
Label	**Description**		
PHY-PA-1	Unamended Coyote brush	5	6/18/14
PHY-PB-1	Unamended Mule-fat	5	6/18/14
PHY-PC-1	Unamended Purple needlegrass	5	6/18/14
PHY-CPA-1	Chelated Coyote brush	5	6/18/14
PHY-FPA-1	Fertilized Coyote brush	5	6/19/14
PHY-SP-1	Sterilized Purple needlegrass	5	6/18/14
PHY-U-1	Unplanted	5	6/19/14
	Total	35	

3.2.8. Phytovolatilization

A microcosm apparatus was constructed to test for the phytovolatilization of COIs. The apparatus consisted of a dual-port Tedlar® bag that was cut open on one end to fit over the plant and around the opening of the microcosm jar. The bag was sealed around the rim of the jar using foam strips overlaid on the outside of the bag and tightened against the jar using cable ties and a hose clamp (Figure 3.7). An aquarium pump was attached to a sorbent tube (Anasorb CSC, coconut charcoal, 6 X 70 mm size, 2-section, 50/100 mg sorbent) which was attached to the inlet port on the Tedlar® bag. The pump would push ambient air through the first sorbent tube and then start to fill up the bag. Once the pressure in the bag built up, any volatilized contaminants would travel through the exit port and be captured in another carbon sorbent tube. All connections were made with Teflon® tubing.

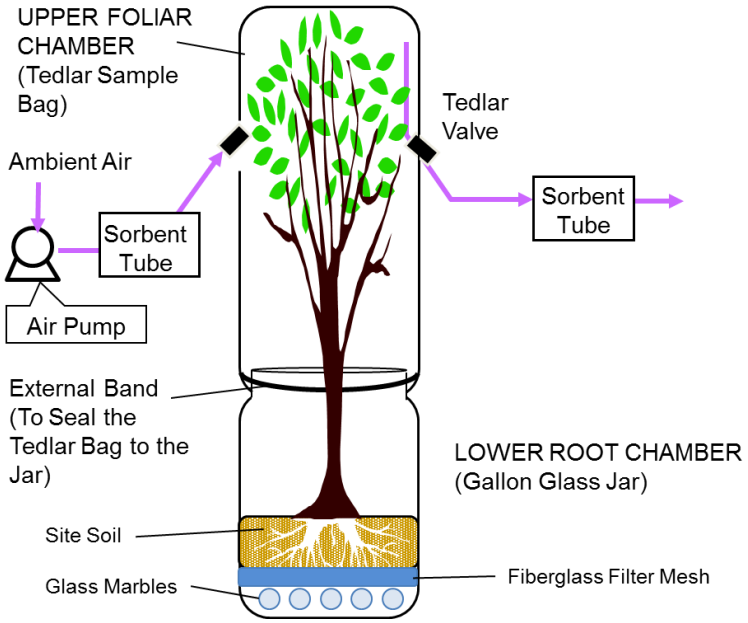


Figure 3.7. Phytovolatilization sampling setup

The target flow rate for this volatilization testing was between 10 mL/min to 200 mL/min based on the NIOSH 1500 air sampling method for hydrocarbons. Microcosms from the different treatments were sampled for 5 days. The flow rate out of the exit sorbent tube was measured once each day using a BIOS DryCal DC-2 Air Flow Calibrator with a model DC-MC-1 flow cell, and then the daily values were averaged (Table 3.6).

Table 3.6. Average flow rates for microcosm phytovolatilization sampling

Treatment	Average Flow Rate (mL/min)
Planted Coyote Brush	75
Planted Mule Fat	74
Planted Needlegrass	<10
Chelated Coyote Brush	21
Unplanted	183
Empty Jar (no soil)	92

The method of extracting contaminants from the sorbent material was a modified version of EPA Standard Method #3550. The sorbent was placed in a 100-mL sample bottle and 2 mL of methylene chloride (MeCl) was added to the sample to extract the COIs. The extraction solution contained 5-g/L hexacosane to serve as an internal recovery standard. MeCl (25 mL) was added to the sample bottle containing the sorbent and sonicated for 3 min at 60,000 Hz using a Sonifier 250 (Branson Ultrasonics Corp., Danbury, Connecticut). Anhydrous sodium sulfate (Na_2SO_4) was added to the extract solution to absorb any moisture. The extract solution was filtered through a 24-cm (diameter) 802 Fluted Grade

Whatman filter paper mounted in a glass funnel with sodium sulfate to remove water from the extracts. The final solution was filtered through a Millipore API 04200 glass fiber filter into a test tube. The extraction was repeated with another 25 mL of MeCl added to the sorbent sample. The total MeCl extract volume was about 50 mL. The extract solution was pipetted into a 2-mL vial with Teflon-lined crimp seal.

Samples from each extract were run through an Agilent Technologies 6890N Gas Chromatograph (splitless inlet) with an Agilent 5975B inert Mass Selective Detector. A 50-m fused silica column 250 microns in diameter was used (Agilent Catalog #19091S-433) with helium as the carrier gas. The oven temperature ramped from 45°C to 275°C at a rate of 12°C per minute and was then held at 275°C for the remainder of the 34-min run time.

3.2.9. Final Microcosm Sampling (211 days)

Final microcosm sampling was conducted on October 15th and 16th, 2014, about 126 days after the midpoint sampling event and 211 days after planting. Foliage, roots and soil were sampled for all of the microcosms. Each of the planted microcosms had 10 replicates available for sampling. Replicates within each treatment group were combined to ensure that there was sufficient root mass for the laboratory analysis. For instance, soil, roots, and foliage from the chelated Coyote Brush replicates CPA01, CPA03, and CPA06 were combined to form a composite sample named CPA01 for the soil, roots, and foliage respectively. Table 3.7 lists the replicate combinations and new sample IDs. The following procedure was used to sample each type of microcosm:

1. The foliage portion of the plant (above ground tissue) was separated from the roots using stainless steel clippers. All of the foliage tissue from each replicate was used to form the composite sample.
2. The foliage was cut into 1-inch pieces and rinsed with DI water to remove any soil. The rinsed foliage was laid onto paper towels to dry for 45 minutes (Figure 3.8) and then placed into a 16-oz sampling jar.
3. Stainless-steel trowels were used to loosen the soil and root mass from the glass microcosm jar. The soil was separated from the roots by hand and placed into a stainless steel bowl. All of the root tissue from each replicate was used to form the composite sample.
4. The roots were rinsed thoroughly with DI water to remove attached soil particles. The rinsed roots were placed onto paper towels to dry for 45 minutes and then placed into a 16-oz sampling jar.
5. The soil from each replicate was combined and homogenized in a stainless steel tray. A composite sample was placed into a 16-oz sampling jar and also in a 4-oz sampling jar for separate EFH analysis.
6. All of the sampling tools were decontaminated between sampling different microcosm treatment types.
7. Duplicate soil samples were taken from the unplanted and sterilized unplanted jars for QA/QC.

8. For transport the 4-oz and 16-oz jars were placed into padded bags and sealed in a quart sized Ziploc bag. An equipment blank was prepared for the sampling event by running DI water over the stainless steel trowels and into the sample jar. The samples were shipped on ice with a holding time of two days allowed. An additional 16-oz jar of soil was collected from the PC, SP, U, and SU microcosm for TRF analysis.

Figure 3.8. Roots and foliage drying

Table 3.7. Replicate combinations for new composite sample IDs

Treatment Type	Replicate Combinations	New Composite Sample ID
Coyote Brush	PA01 and 06; PA02 and 07; PA03 and 08; PA04 and 09; PA05 and 10	PA01; PA02; PA03; PA04; PA05
Mule Fat	PB01 and 06; PB02 and 07; PB03 and 08; PB04 and 09; PB05 and 10	PB01; PB02; PB03; PB04; PB05
Purple Needlegrass	PC02 and 03 and 05 and 09 and 10; PC01 and 04 and 06 and 07 and 08	PC01; PC02
Fertilized Coyote Brush	FPA01 and 06; FPA02 and 07; FPA03 and 08; FPA04 and 09; FPA05 and 10	FPA01; FPA02; FPA03; FPA04; FPA05
Chelated Coyote Brush	CPA01 and 03 and 06; CPA02 and 04 and 07; CPA05 and 08 and 09	CPA01; CPA02; CPA03
Sterilized Purple Needlegrass	SP02 and 03 and 05 and 09 and 10; SP01 and 04 and 06 and 07 and 08	SP01; SP02

3.2.10. Terminal Restriction Fragment (TRF) Analysis

TRF is a molecular method that provides a genetic snapshot of microbial communities. This study used the method used by Kaplan and Kitts (2004). DNA was extracted from soil by washing the cells out of soil samples, filtering them, and lysing them. The DNA extracted from the soil was then amplified using PCR and digested by restriction enzymes, creating a wide range of DNA fragment sizes which were then analyzed on a fragment analyzer to allow identification of the microbial communities.

3.2.11. Statistical Methods for Microcosm Analysis

All statistical analyses were performed using Minitab 16 Statistical Software (except for TRF statistical analysis). One-way analysis of variance (ANOVA) was performed to compare the contaminant soil concentrations between treatments at Day 85 or Day 211 of the experiment. The response variable was the contaminant concentration, the factor was the treatment type, and the confidence level was 95%. Tukey comparisons were performed with a family error rate set to 5%.

Two sample t-tests were performed to compare the contaminant concentrations in soil at Day 0 and Day 211. The response variable was contaminant concentration, the factor was the sampling day, and the confidence level was 95%.

Two sample t-tests were also performed to compare the contaminant concentrations in the roots or foliage of different planted microcosm treatments. The response variable was contaminant concentration, the factor was the type of treatment, and the confidence level was 95%.

4.0. Results

4.1. Phase I: Field Screening Results

The field screening was designed to identify which plant species extracted contaminants into their roots or foliage in the field. The soil in contact with the roots of each plant specimen was sampled to give an indication of the contaminant concentrations that the plants were exposed to. However, the root systems were usually very extensive and soil was only collected from some of the areas in contact with the roots. The control locations were selected based on historical sampling data to identify areas where contaminant levels in the soil were non-detect or at background levels. Some of the control locations were discovered to contain COIs while some of the contaminated soil were non-detect. Any location with non-detects or background were utilized as controls, irrespective the area. The following sections describe the screening results for each contaminant type.

4.1.1. Petroleum Hydrocarbon Phytoextraction in the Field

The field results for petroleum hydrocarbon phytoextraction were similar for all plant species screened. Extractable fuel hydrocarbon (EFH) concentrations in the soil around the specimens were 200 mg/kg or lower, while many of the root concentrations were above 1,000 mg/kg and many foliage concentrations were above 4,000 mg/kg (Figures 4.1 to 4.9). For example, for Palmer's Goldenbush (PG), EFH concentrations in the foliage were as high as 59,000 mg/kg while the soil concentration was only 4.7 mg/kg (Figure 4.1). It is likely that phytogenic compounds are contributing to the relatively high EFH concentrations in the plant tissue of all the species. The soil contained hydrocarbons in the C21 to C40 carbon range while the roots and foliage of the plants showed concentrations of hydrocarbons in the C5 through C40 range. The EFH analysis involves summing all the peaks over a wide range of elution times, and it is likely that many types of natural organic material (NOM) are being counted in the EFH analysis. Further, even the plants growing in control soils with little or no EFH in the soil exhibited high EFH concentrations in the roots and foliage, so it is extremely unlikely that the compounds counted as EFH in the plant tissue were from petroleum hydrocarbons extracted from the soil.

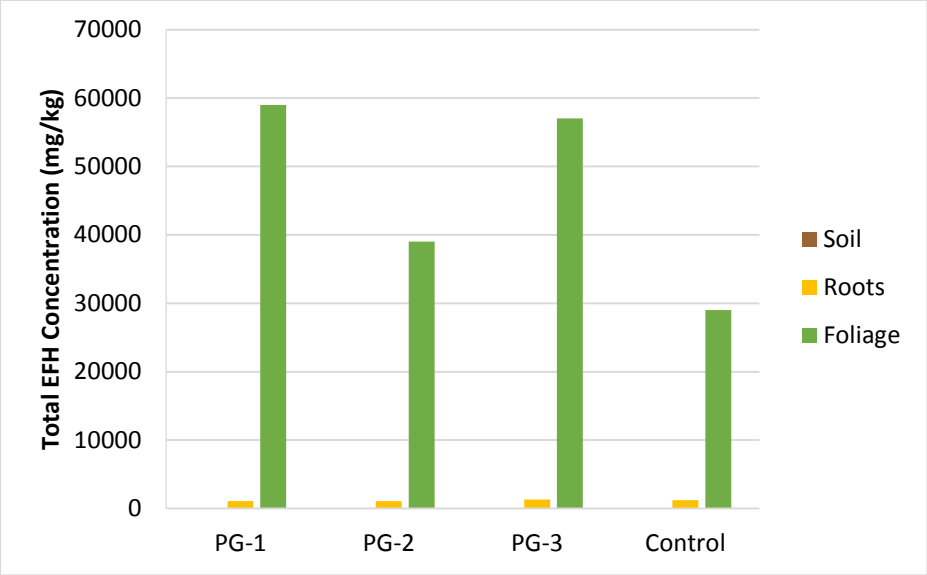


Figure 4.1. Total EFH concentrations for field samples of Palmer's Goldenbush

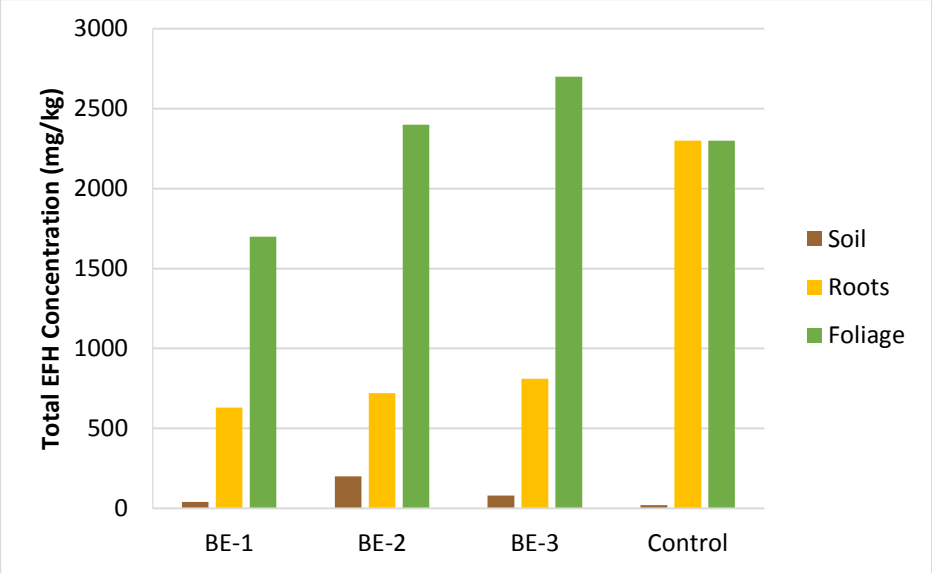


Figure 4.2. Total EFH concentrations for field samples of Blue Elderberry

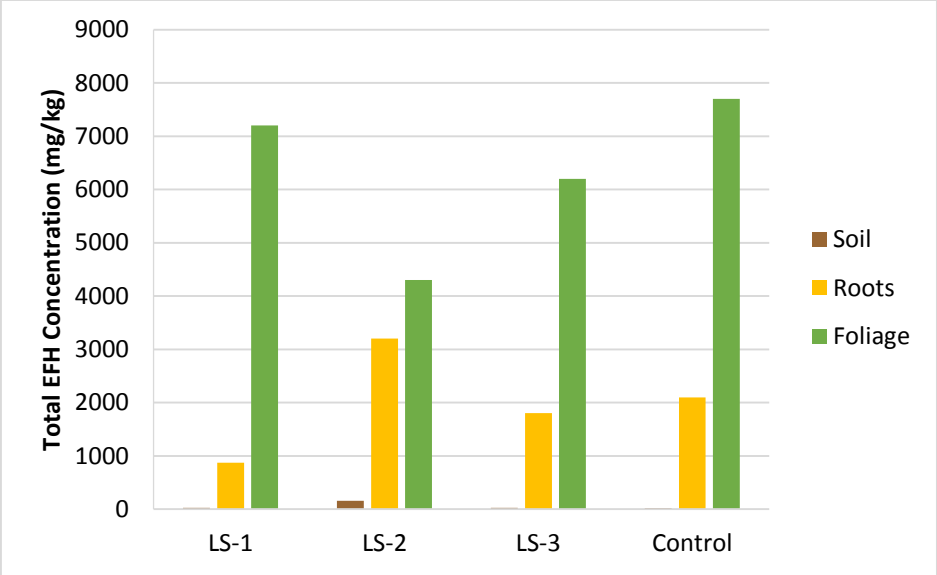


Figure 4.3. Total EFH concentrations for field samples of Laurel Sumac

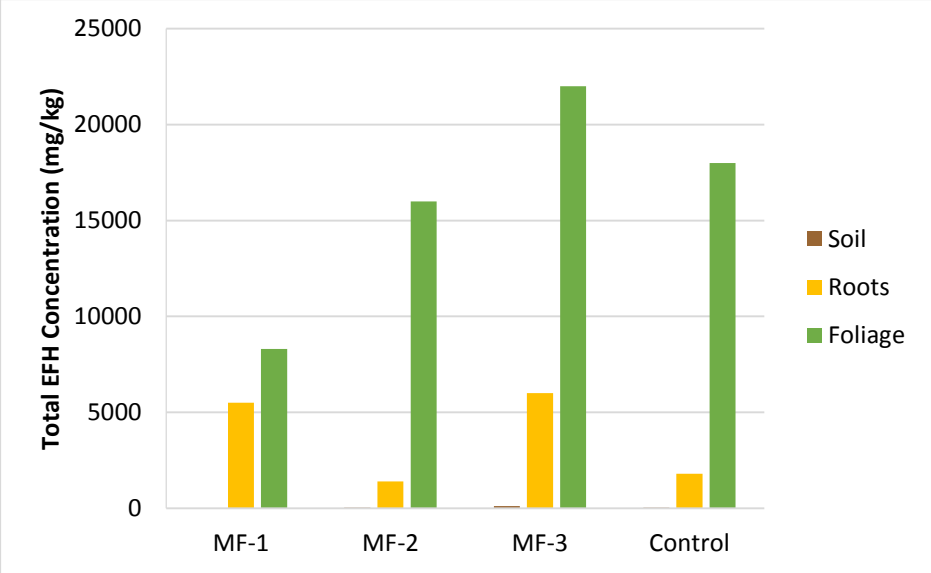


Figure 4.4. Total EFH concentrations for field samples of Mule Fat

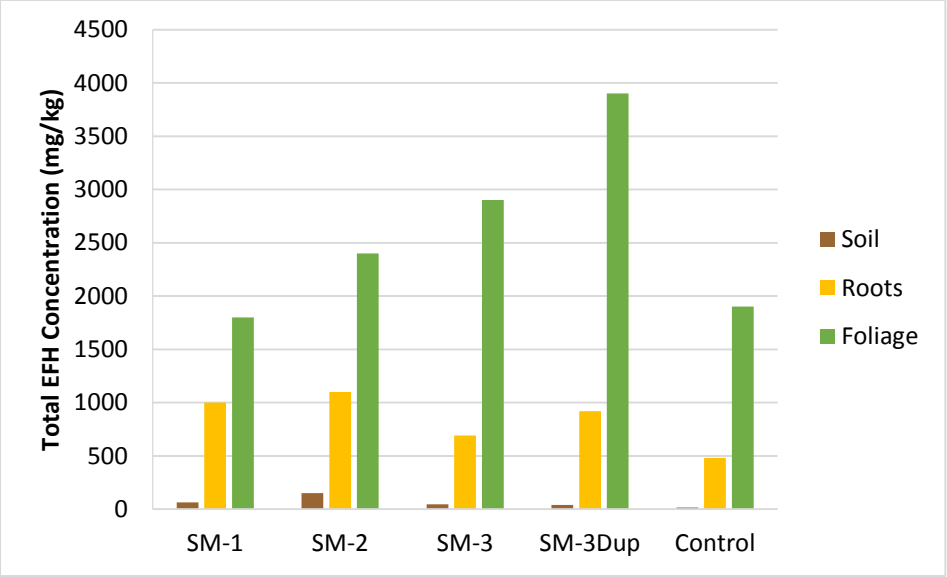


Figure 4.5. Total EFH concentrations for field samples of Summer Mustard

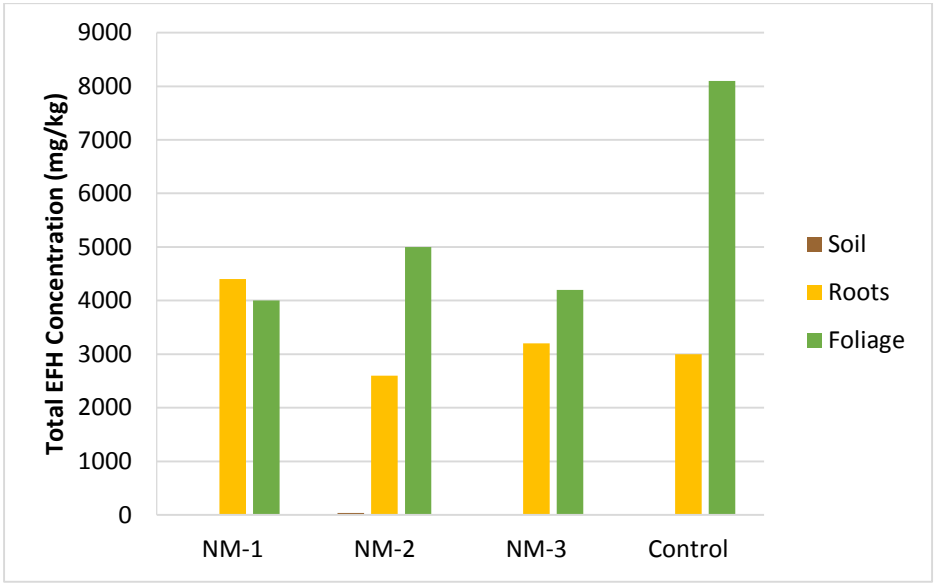


Figure 4.6. Total EFH concentrations for field samples of Narrowleaf Milkweed

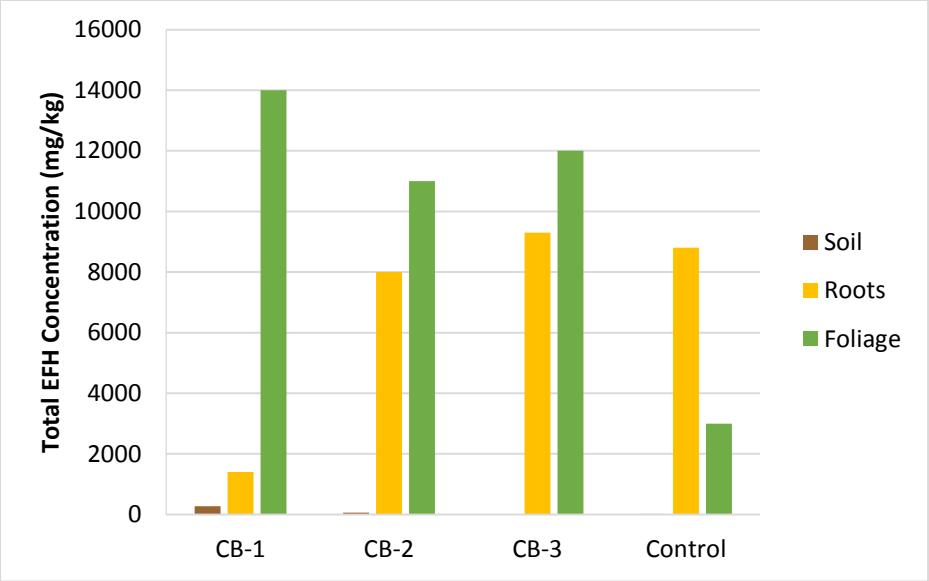


Figure 4.7. Total EFH concentrations for field samples of Coyote Brush

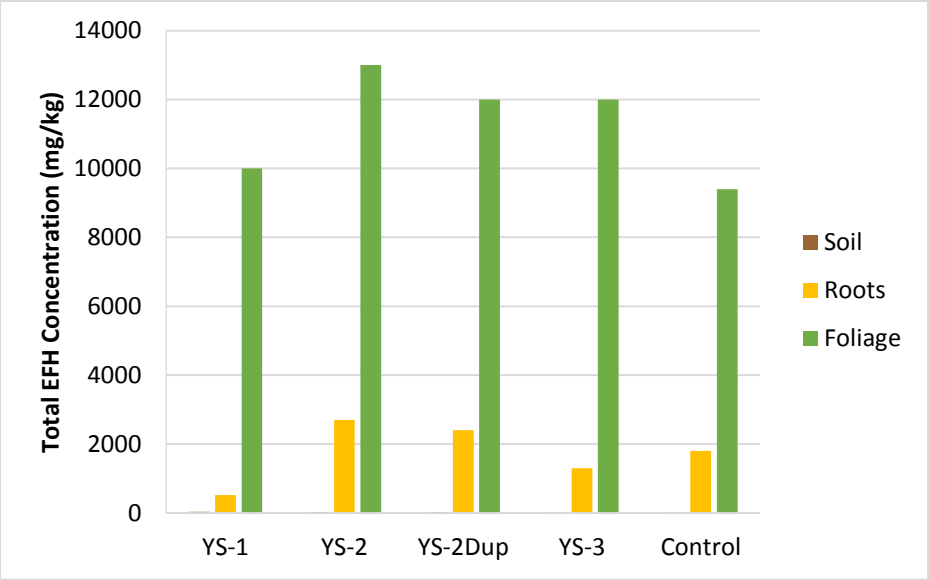


Figure 4.8. Total EFH concentrations for field samples of Yerba Santa

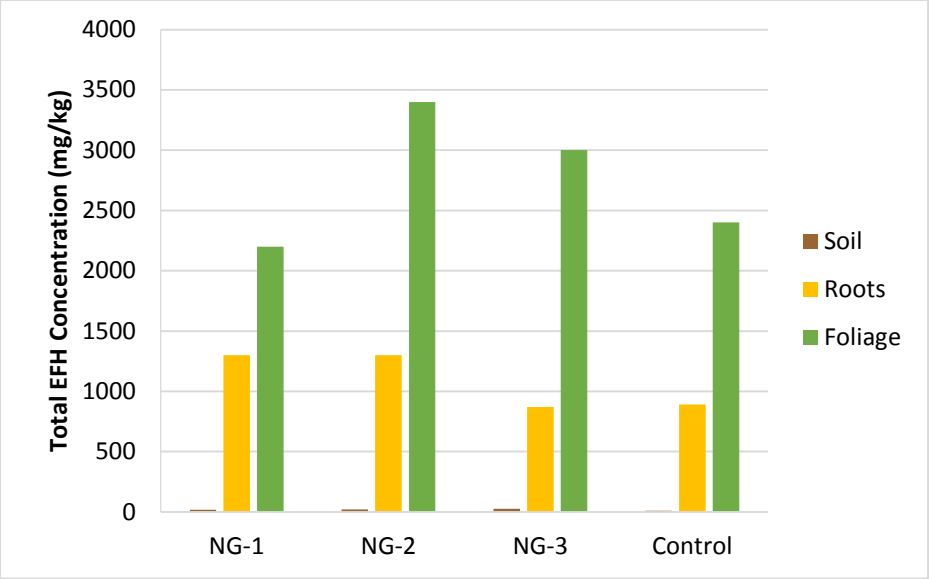


Figure 4.9. Total EFH concentrations for field samples of Purple Needlegrass

4.1.2. Polyaromatic Hydrocarbon Phytoextraction in the Field

PAHs were detected in the roots and foliage of some species (Figures 4.10 to 4.18). Blue Elderberry (BE), Yerba Santa (YS), and Purple Needlegrass (NG) showed the most apparent PAH uptake (Figures 4.10 to 4.12). One of the Blue Elderberry specimens (BE-2) had very high PAH concentrations in the soil, and for this specimen the total PAH concentration in the roots was 1,740 µg/kg (Figure 4.10). The PAHs observed in this specimen do not appear to be phytogenic since the other three plant specimens sampled were non-detect for PAHs in the plant tissue (as well as the soil). The PAHs observed in the Blue Elderberry roots were: benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene. dibenzo(a,h)anthracene, fluoranthene, fluorene, and indeno(1,2,3-cd)pyrene which were all also present in the soil. No PAHs were observed in the roots or foliage of Palmer's Goldenbush.

Concentrations of PAHs in the roots of one specimen of Yerba Santa (YS-2) were about 6-8x higher than that of the surrounding soil (Figure 4.11), indicating possible bioconcentration. These PAHs also do not appear to be phytogenic since the other specimens of the same species did not have any PAHs in the plant tissue. However, the PAH measured in the roots was anthracene which was not detected in the nearby soil.

PAHs were detected in the roots of one Purple Needlegrass specimen (700 µg/kg) and the foliage of one specimen (4,400 µg/kg) as well (Figure 4.12). Specimen NG-1 showed benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, fluoranthene, indeno(1,2,3-cd)pyrene, and pyrene present in the foliage. Specimen NG-2 had benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene, chrysene, indeno(1,2,3-cd)pyrene, and pyrene in the roots. Most of these PAHs were also observed in the soil at lower concentrations.

PAHs were present in the foliage of all of the Mule Fat specimens, including the control, despite an absence PAHs in soil (Figure 4.13). This indicates that the PAHs observed in Mule Fat are most likely phytogenic compounds that are eluting at similar times as PAHs. None of the Mule Fat specimens showed PAHs in the roots. The foliage of one Mule Fat specimen (MF-1) had benzo(a)anthracene and a small amount of benzo(e)pyrene. The foliage of Specimen MF-3 had only benzo(a)anthracene. MF- 2 and the control Mule Fat growing in uncontaminated soil had only chrysene. These differences indicate that the type of phytogenic compound produced may be different between Mule Fat specimens.

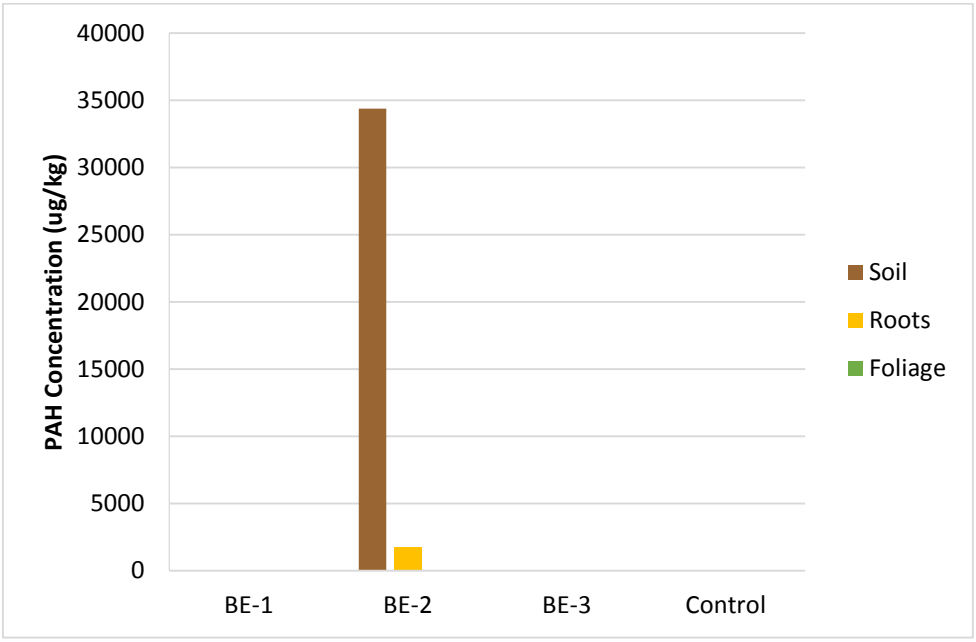


Figure 4.10. Total PAH concentrations for field samples of Blue Elderberry

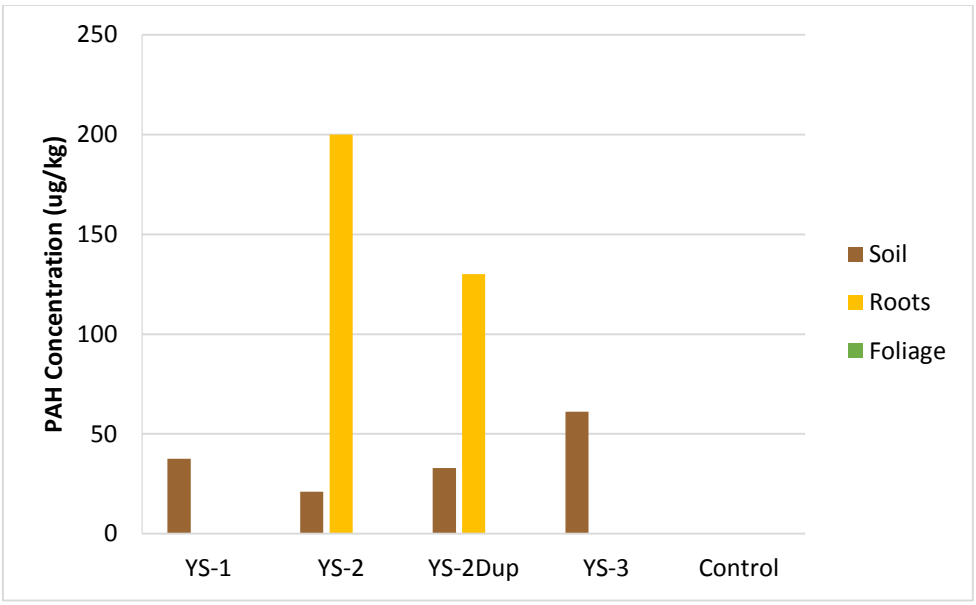


Figure 4.11. Total PAH concentrations for field samples of Yerba Santa

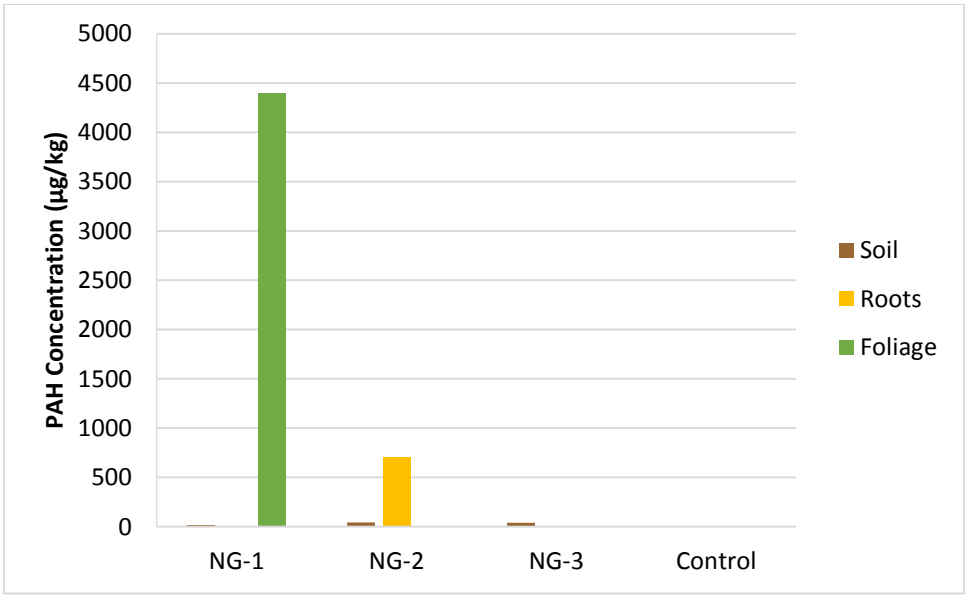


Figure 4.12. Total PAH concentrations for field samples of Purple Needlegrass

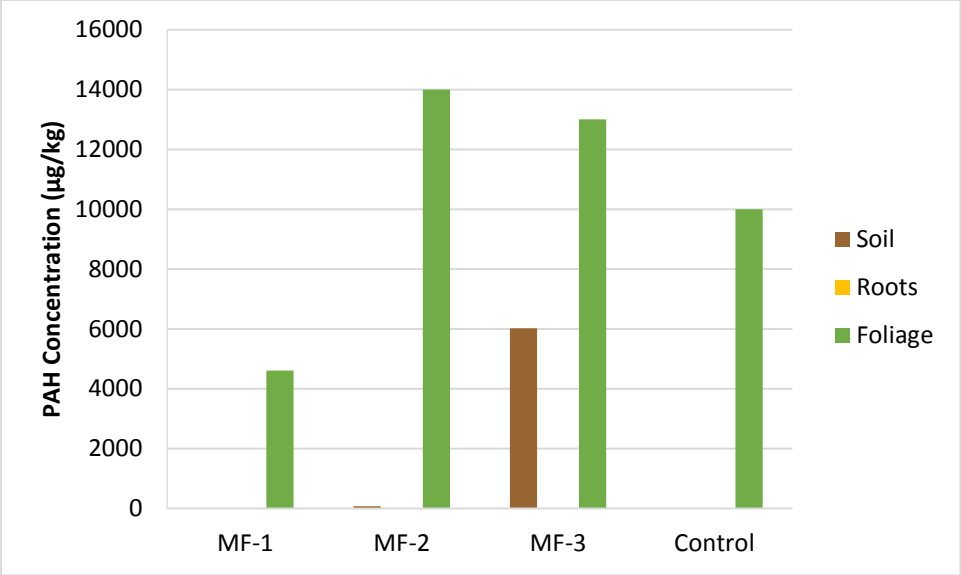


Figure 4.13. Total PAH concentrations for field samples of Mule Fat

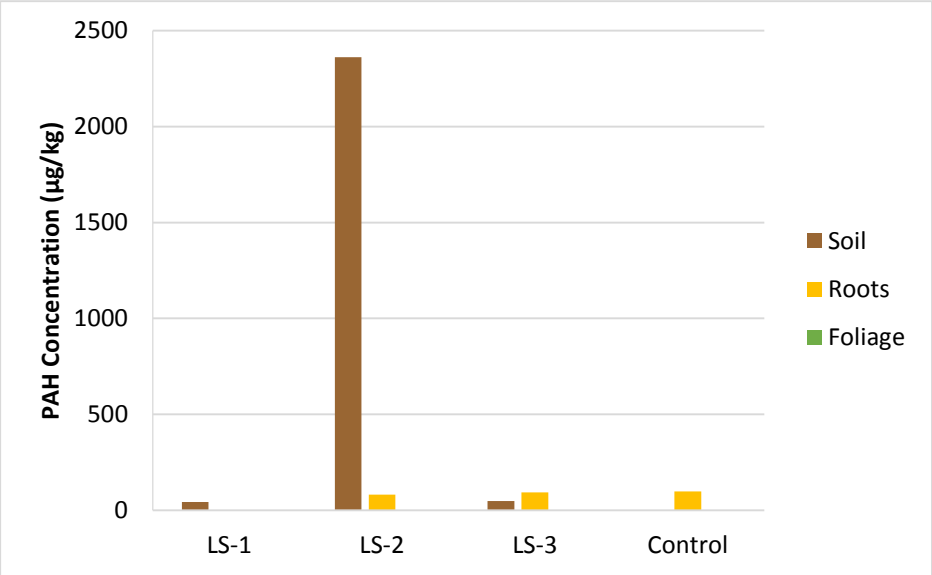


Figure 4.14. Total PAH concentrations for Laurel Sumac

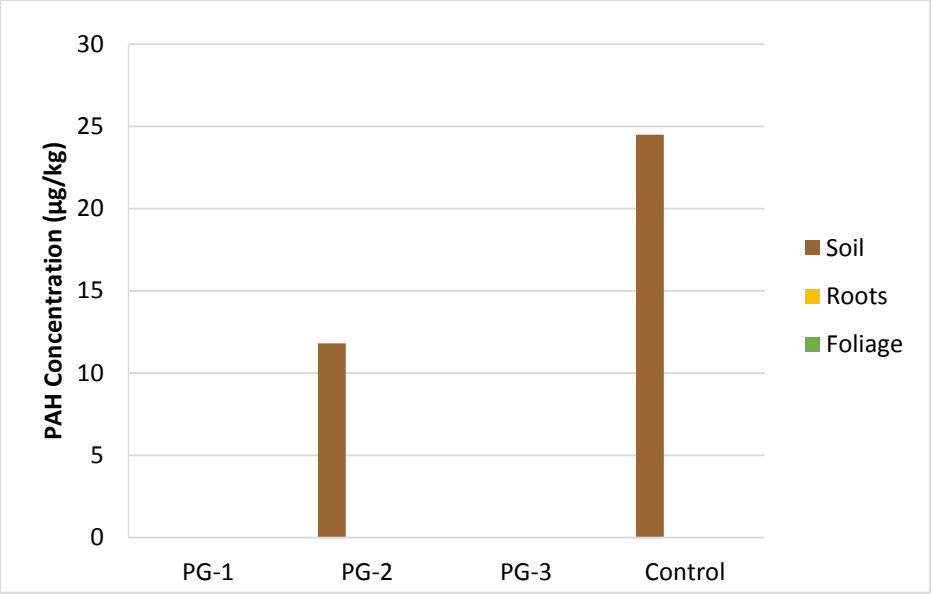


Figure 4.15. Total PAH concentrations for field samples of Palmer's Goldenbush

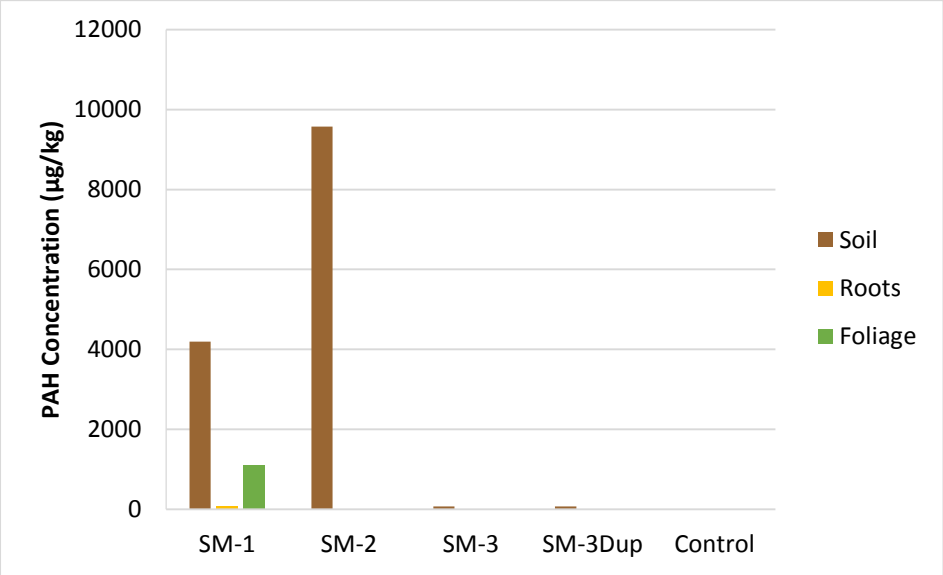


Figure 4.16. Total PAH concentrations for field samples of Summer Mustard

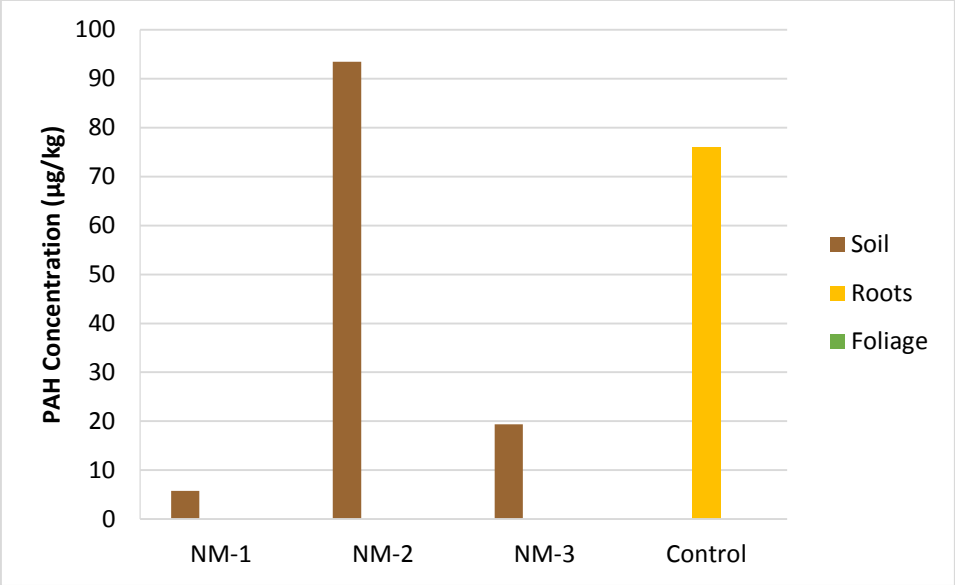


Figure 4.17. Total PAH concentrations for field samples of Narrowleaf Milkweed

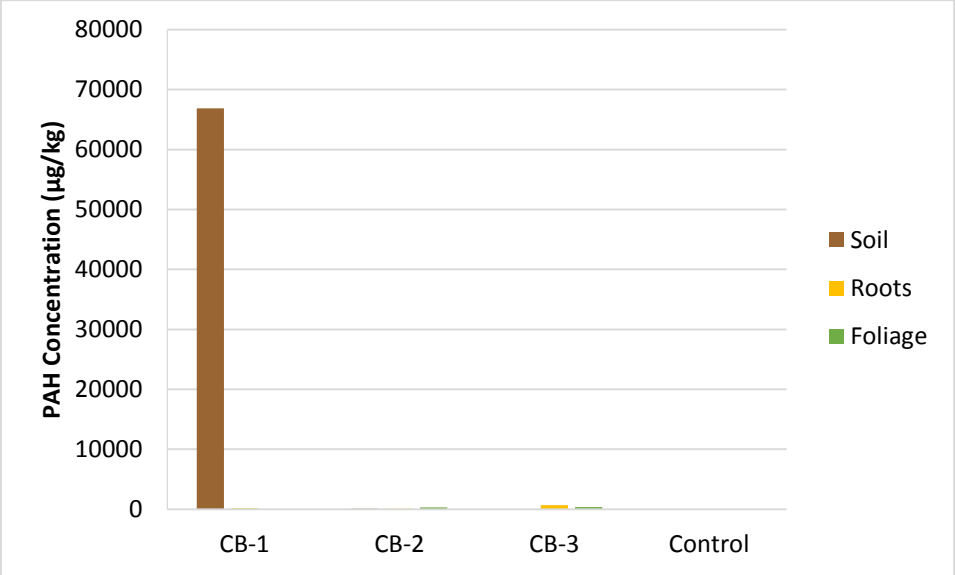


Figure 4.18. Total PAH concentrations for field samples of Coyote Brush

4.1.3. Polychlorinated Biphenyl Phytoextraction in the Field

None of the plants species showed any phytoextraction of PCBs from the soil. All of the root and foliage had PCB concentrations below the detection limit of 50-200 µg/kg. PCBs were not detected in the soils associated with Palmer's Goldenbush and Purple Needlegrass specimens, so it is not known if these species could phytoextract PCBs if they were present in the soil.

4.1.4. Chlorinated Dioxins/Furans Phytoextraction in the Field

Blue Elderberry, Palmer's Goldenbush, Yerba Santa, and Purple Needlegrass showed potential uptake of chlorinated dioxins/furans in either their roots, foliage, or both (Figures 4.19 to 4.22). Purple Needlegrass had a total dioxin/furan concentration of 2,200 ng/kg in the roots of one specimen, and a total dioxin/furan concentration of 690 ng/kg in the foliage of another specimen (Figure 4.19). The amount of dioxin/furan uptake by Purple Needlegrass appeared to be positively correlated to the concentration of dioxins/furans in the nearby soil. Blue Elderberry had a dioxin/furan concentration of 1,000 ng/kg in the roots of specimen BE-2 (Figure 4.20). Dioxin/furan concentrations in soil associated with this specimen were very high. Yerba Santa had a concentration of 420 ng/kg in the roots of one specimen (YS-2Dup) and a concentration of 900 ng/kg in the foliage of another specimen (YS-3) (Figure 4.21). All of the controls except for Palmer's Goldenbush had very small dioxin/furan concentrations in the soil (Figure 4.22). The congeners that were found to make up most of the total dioxin/furan concentrations in the soil were octachlorodibenzo-p-dioxin and 1, 2, 3, 4, 6, 7, 8-heptachlorodibenzo-p-dioxin. These highly chlorinated dioxins were also the primary congeners seen in the plant roots and foliage of the screened species (Figures 4.19 to 4.27).

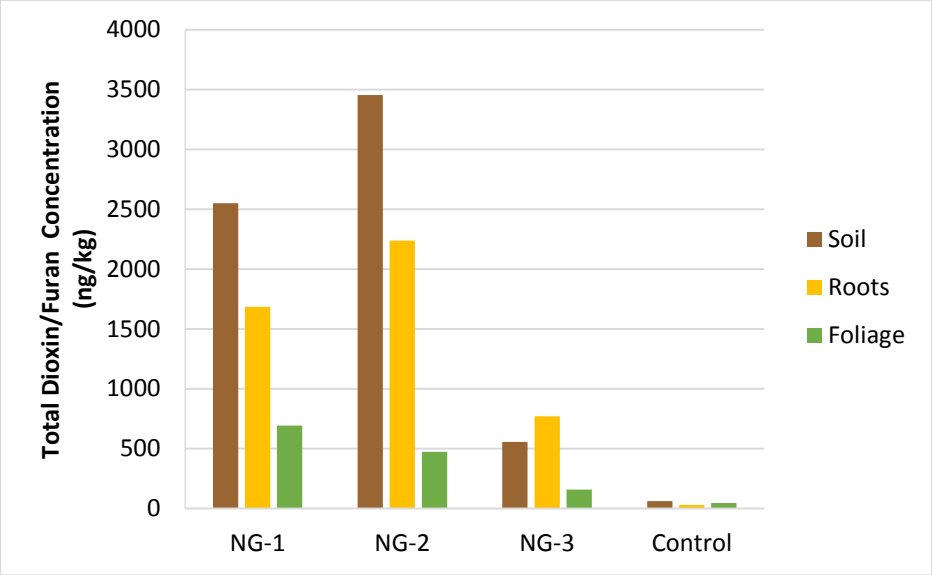


Figure 4.19. Total dioxin/furan concentrations for field samples of Purple Needlegrass

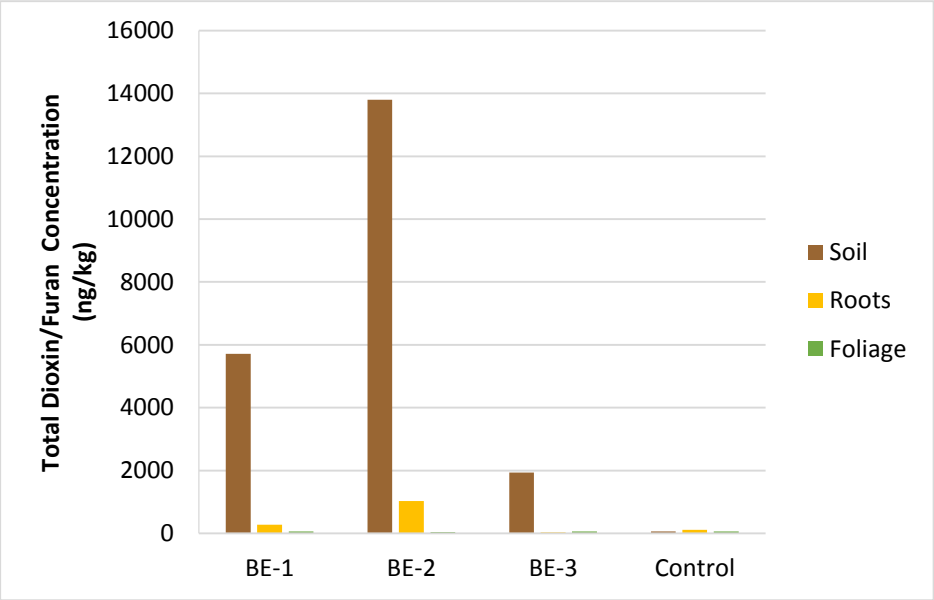


Figure 4.20. Total dioxin/furan concentrations for field samples of Blue Elderberry

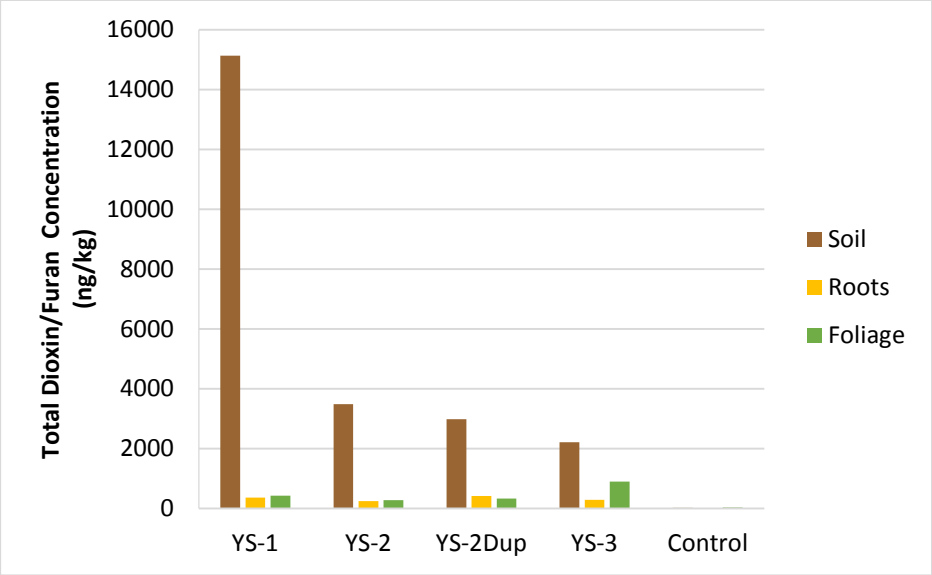


Figure 4.21. Total dioxin/furan concentrations for field samples of Yerba Santa

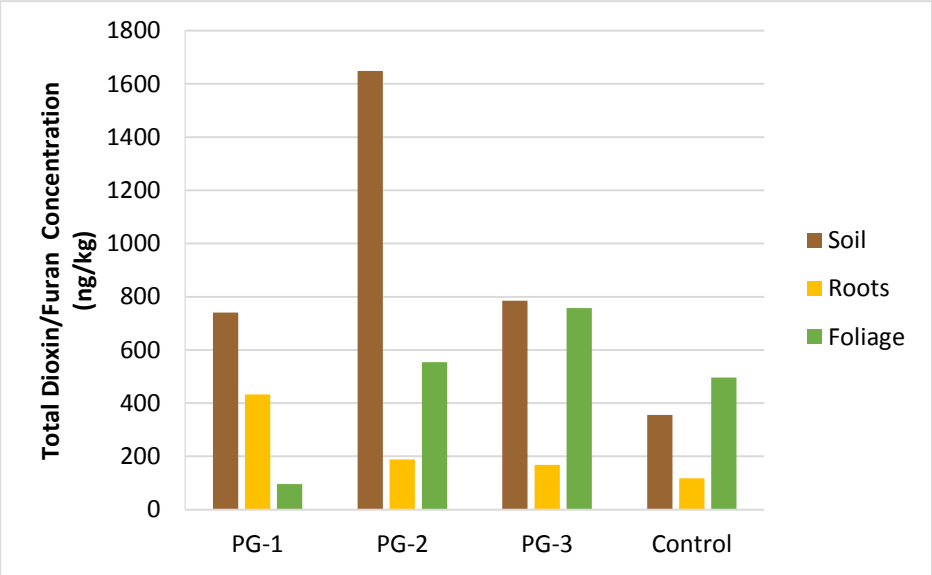


Figure 4.22. Total dioxin/furan concentrations for field samples of Palmer's Goldenbush

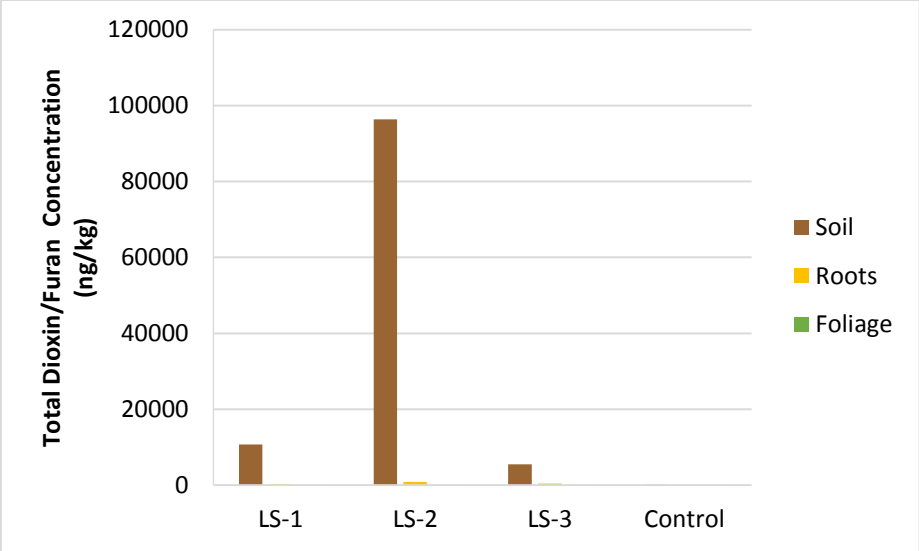


Figure 4.23. Total dioxin/furan concentrations for field samples of Laurel Sumac

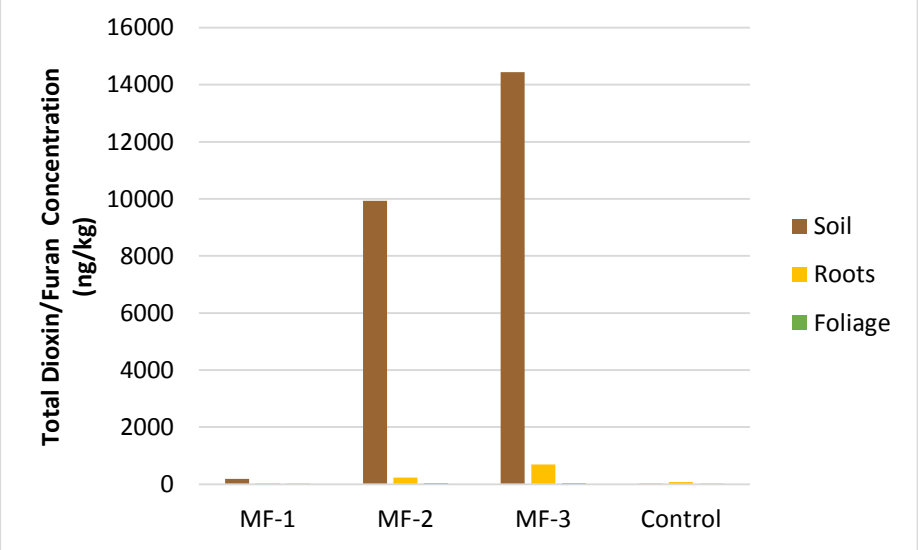


Figure 4.24. Total dioxin/furan concentrations for field samples of Mule Fat

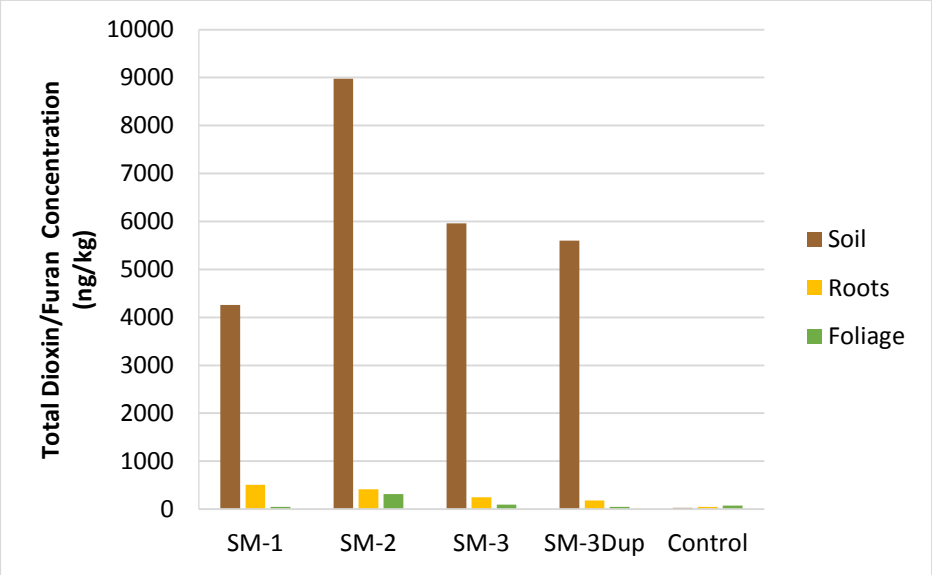


Figure 4.25. Total dioxin/furan concentrations for field samples of Summer Mustard

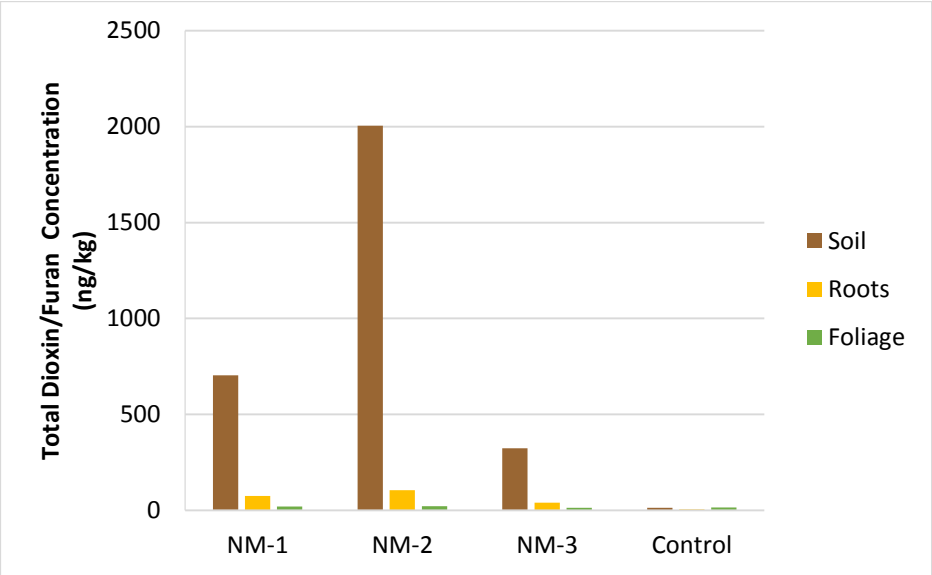


Figure 4.26. Total dioxin/furan concentrations for field samples of Narrowleaf Milkweed

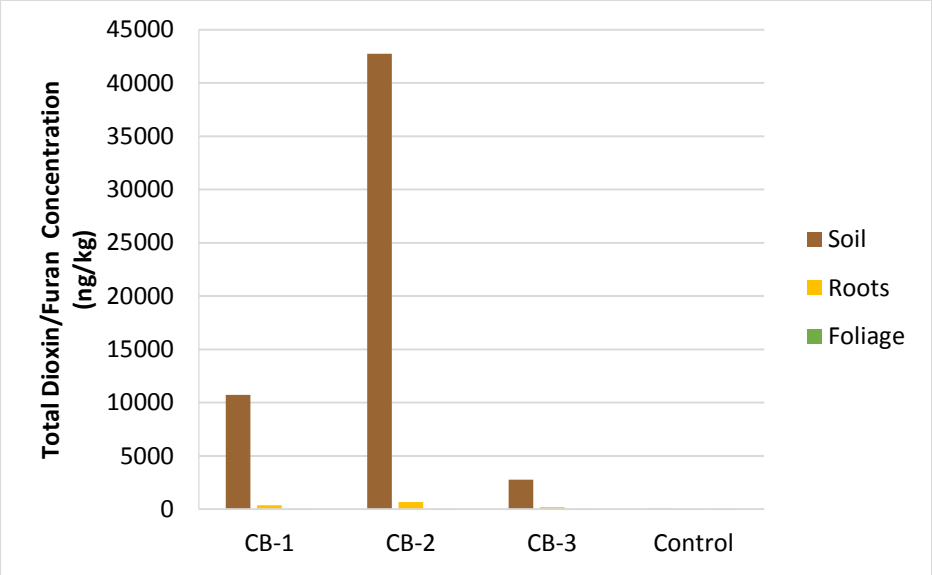


Figure 4.27. Total dioxin/furan concentrations for field samples of Coyote Brush

4.1.5. Mercury Phytoextraction in the Field

Root and foliage mercury concentrations were below the detection limit of 0.1 mg/kg for all species tested in the field. Mercury was present in most of the soil samples at concentrations ranging from 0.057 mg/kg to 0.27 mg/kg, but mercury was below the detection limits in soil associated with Palmer's Goldenbush, Narrowleaf Milkweed, and Purple Needlegrass.

4.1.6. Silver Phytoextraction in the Field

Silver was observed in the roots of all plant species except for Palmer's Goldenbush and Purple Needlegrass (Figures 4.28 to 4.36). One of the Laurel Sumac specimens (LS-3) showed the highest uptake of silver into the roots with a concentration of 7.3 mg/kg (Figure 4.28). Summer Mustard (SM) was the only species that showed uptake of silver into the foliage, and this was at a concentration of 0.41 mg/kg in one specimen (SM-3) (Figure 4.29).

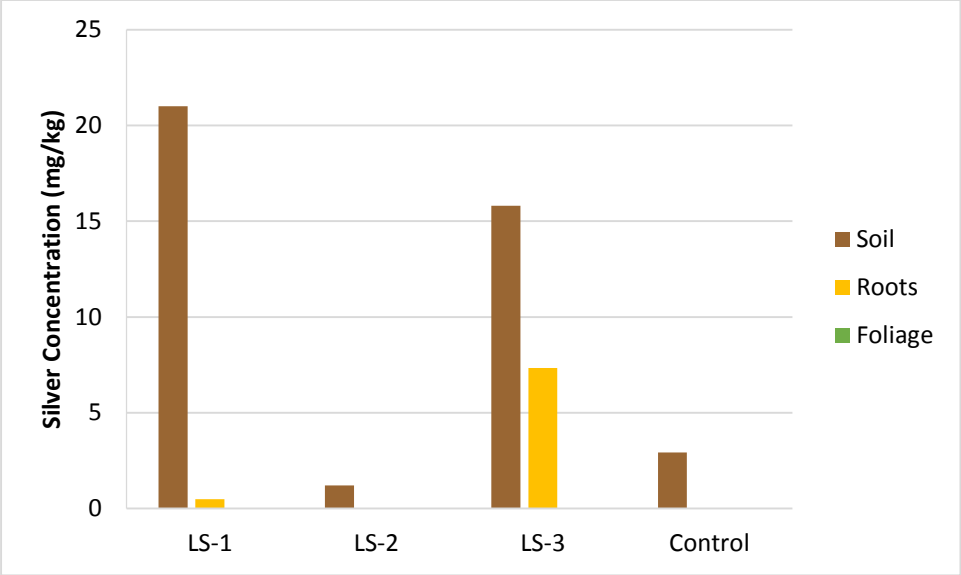


Figure 4.28. Silver concentrations for field samples of Laurel Sumac

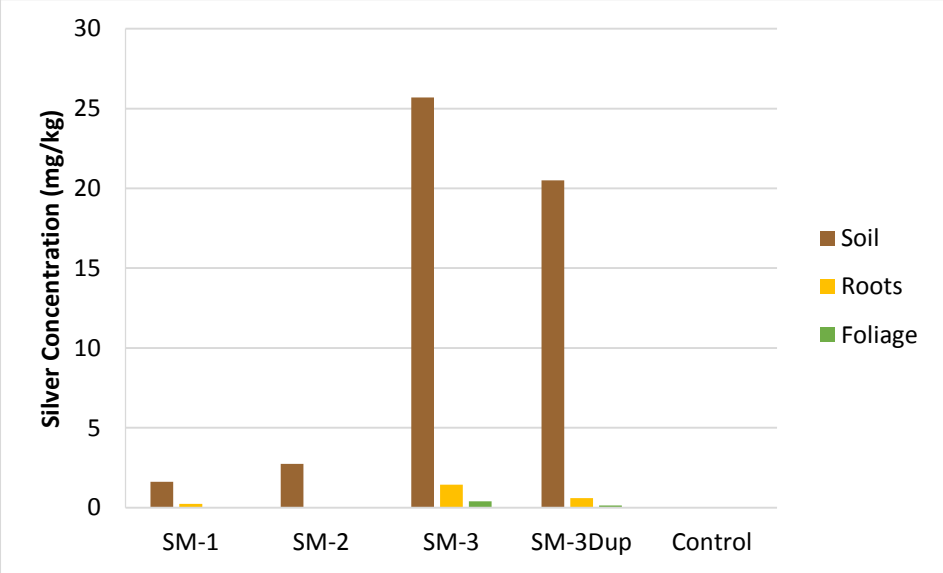


Figure 4.29. Silver concentrations for field samples of Summer Mustard

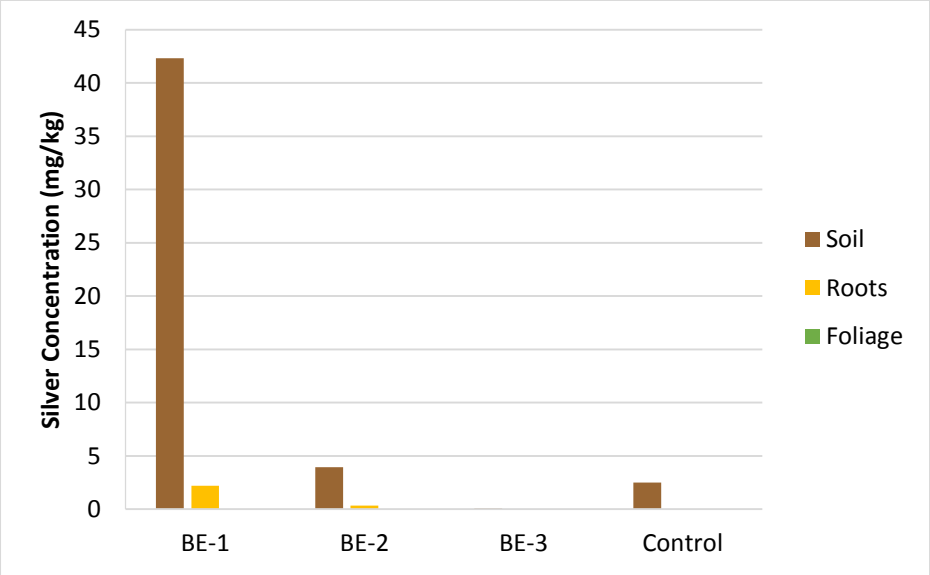


Figure 4.30. Silver concentrations for field samples of Blue Elderberry

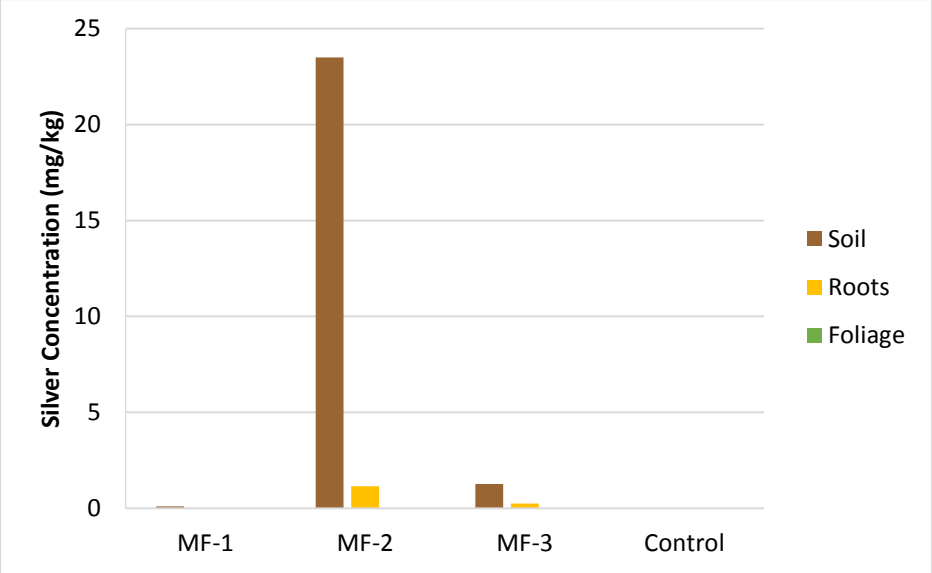


Figure 4.31. Silver concentrations for field samples of Mule Fat

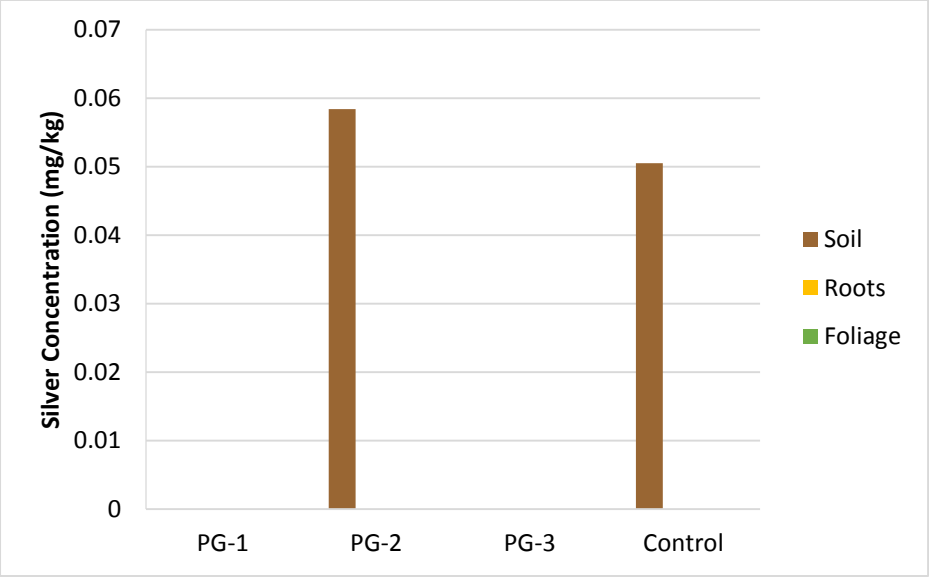


Figure 4.32. Silver concentrations for field samples of Palmer's Goldenbush

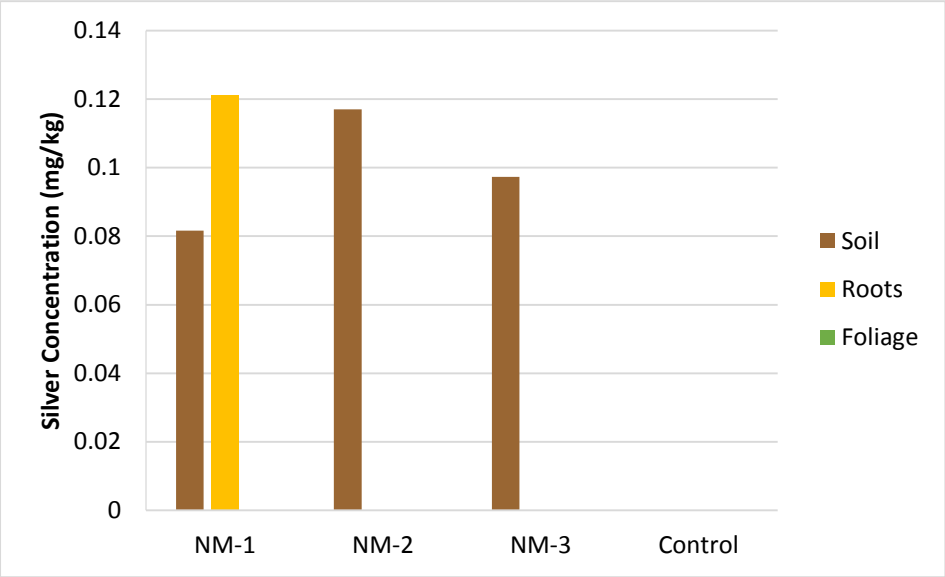


Figure 4.33. Silver concentrations for field samples of Narrowleaf Milkweed

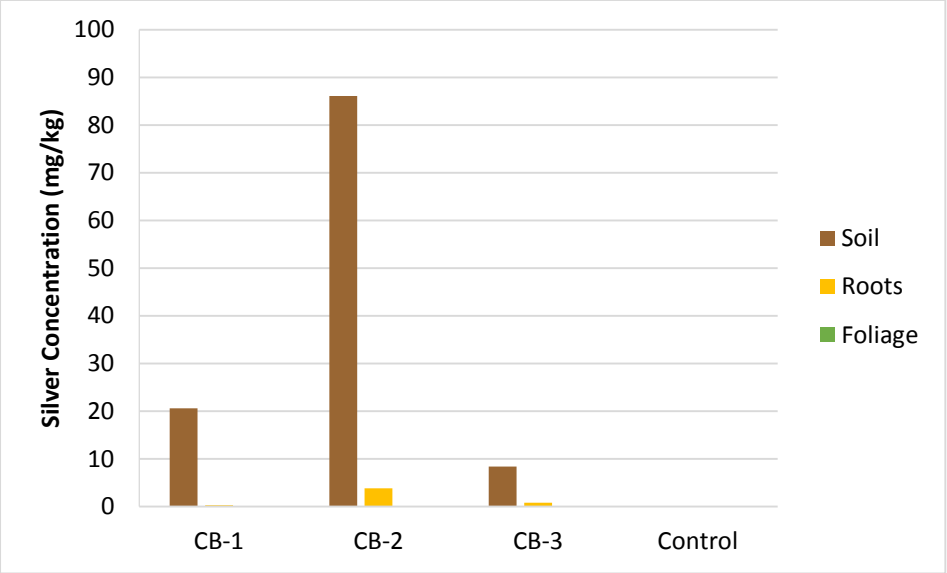


Figure 4.34. Silver concentrations for field samples of Coyote Brush

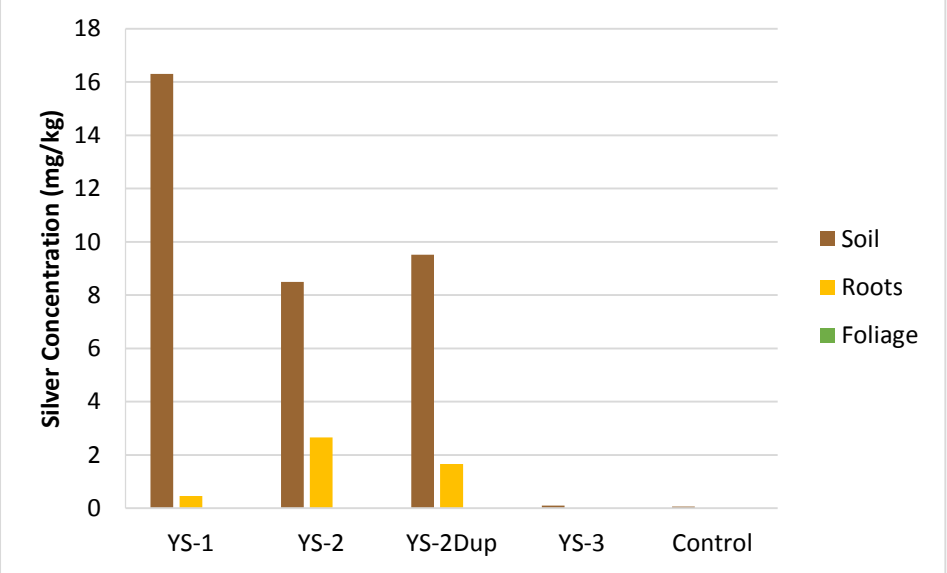


Figure 4.35. Silver concentrations for field samples of Yerba Santa

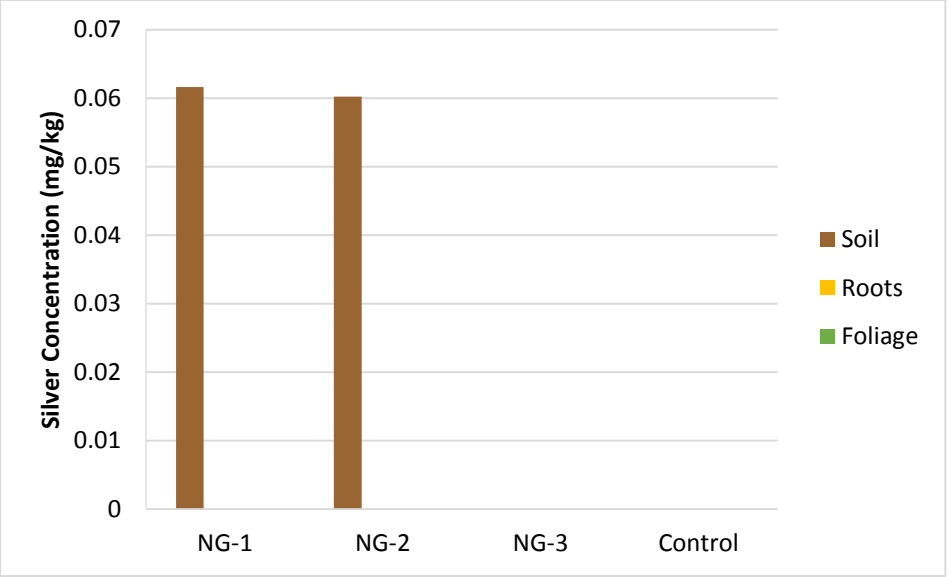


Figure 4.36. Silver concentrations for field samples of Purple Needlegrass

4.1.7. Cadmium Phytoextraction in the Field

Most of the soil concentrations for cadmium were at or below the action level of 0.7 mg/kg (Figures 4.37 to 4.45). At these low levels Mule Fat showed the greatest uptake of cadmium in the field screening. One specimen (MF-2) had a concentration of 1.8 mg/kg in the roots, and a concentration of 3.6 mg/kg in the foliage compared to a soil concentration of 1.0 mg/kg (Figure 4.37). Coyote Brush showed uptake and translocation of cadmium with soil levels below and above 0.7 mg/kg. Specimens CB-1, CB-3, and the control showed higher concentrations of cadmium in the plant tissue than in the soil (Figure 4.38).

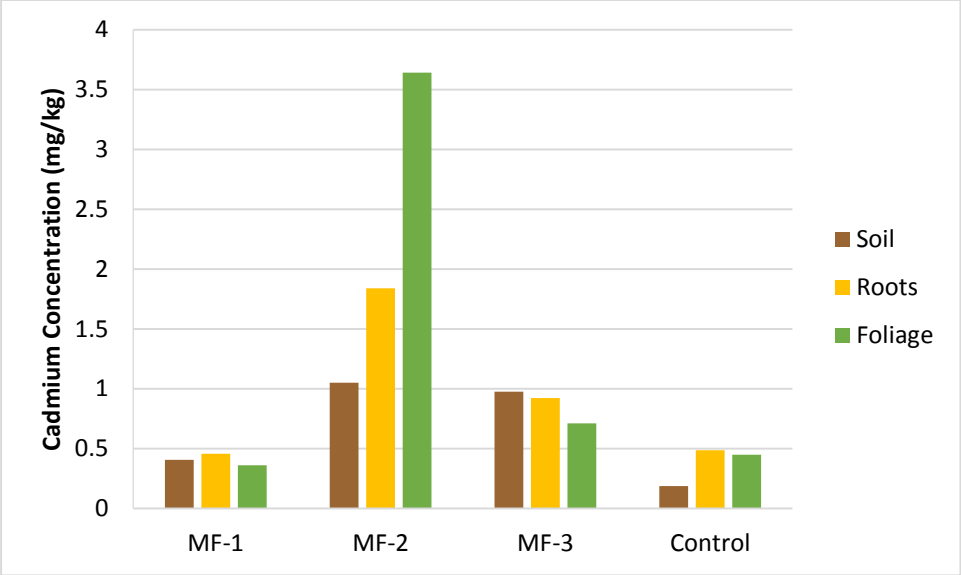


Figure 4.37. Cadmium concentration for field samples of Mule Fat

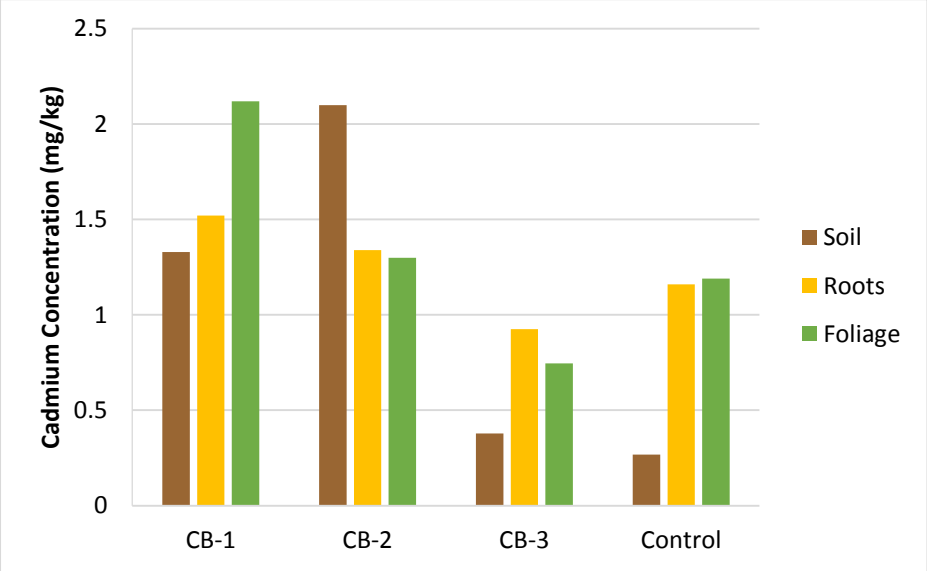


Figure 4.38. Cadmium concentrations for field samples of Coyote Brush

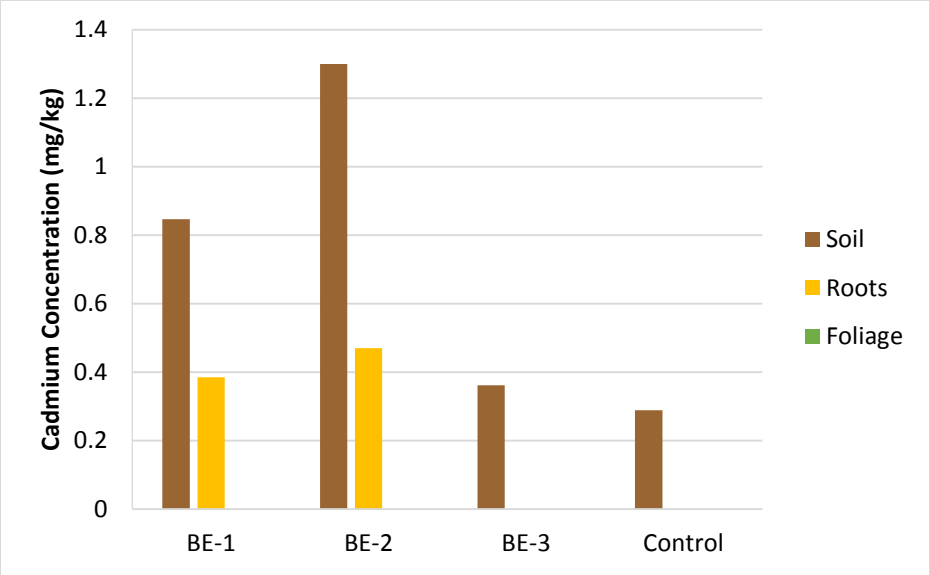


Figure 4.39. Cadmium concentrations for field samples of Blue Elderberry

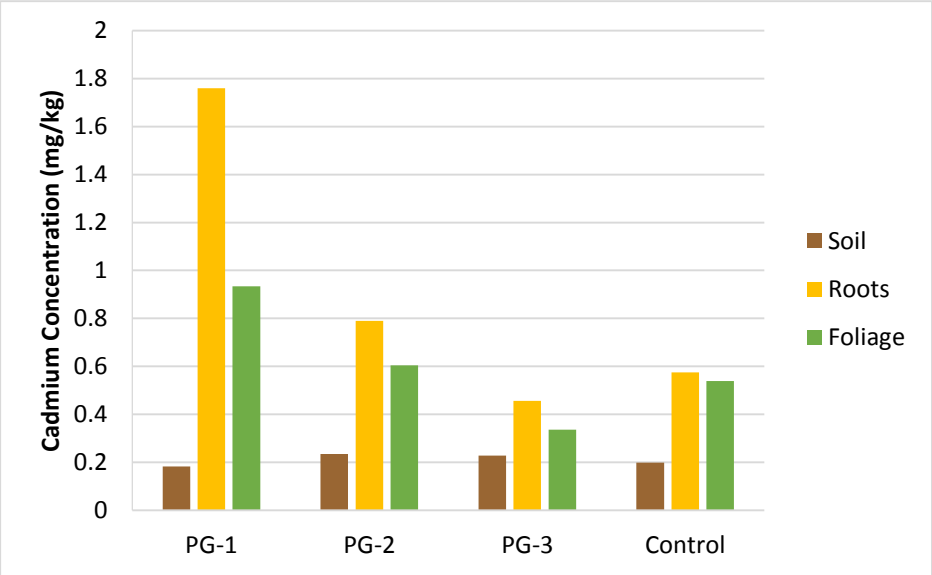


Figure 4.40. Cadmium concentrations for field samples of Palmer's Goldenbush

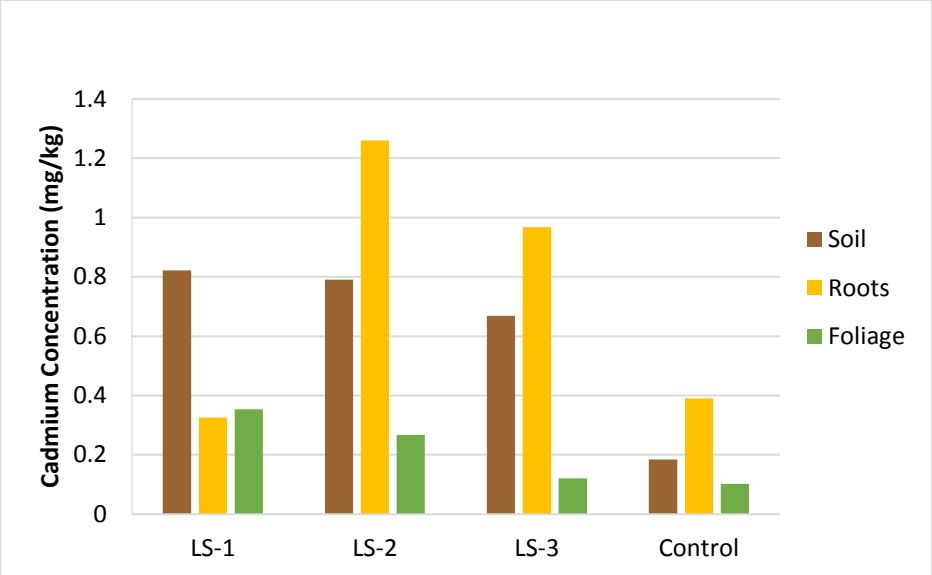


Figure 4.41. Cadmium concentrations for field samples of Laurel Sumac

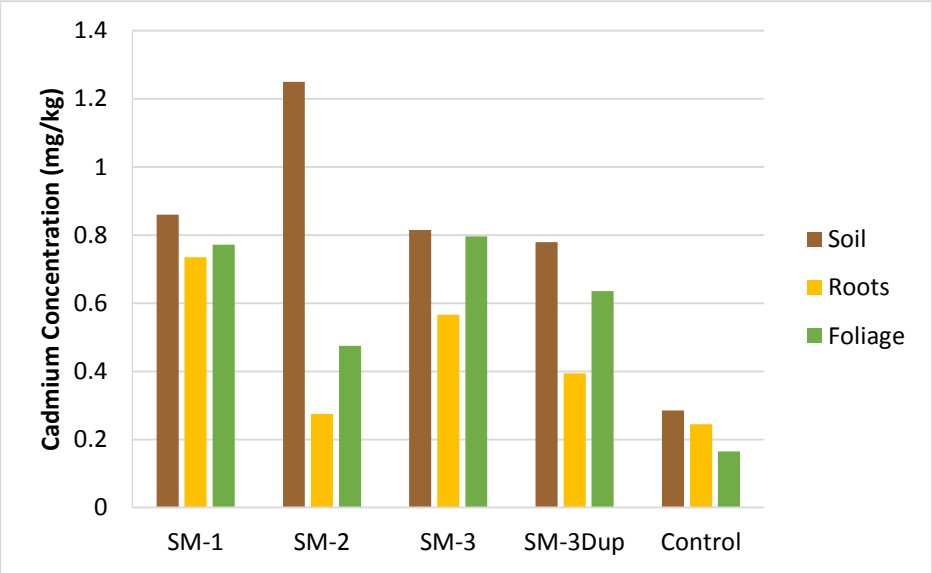


Figure 4.42. Cadmium concentrations for field samples of Summer Mustard

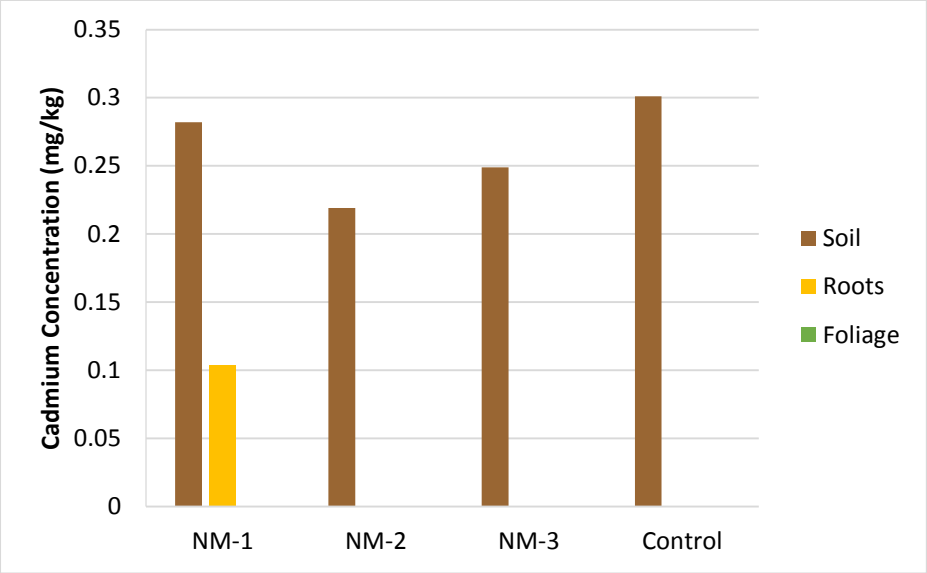


Figure 4.43. Cadmium concentrations for field samples of Narrowleaf Milkweed

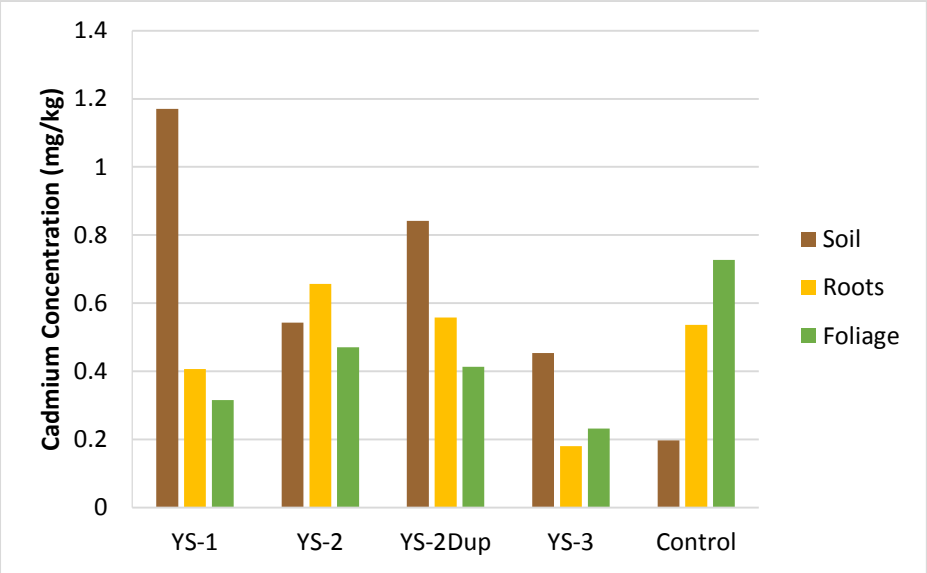


Figure 4.44. Cadmium concentrations for field samples of Yerba Santa

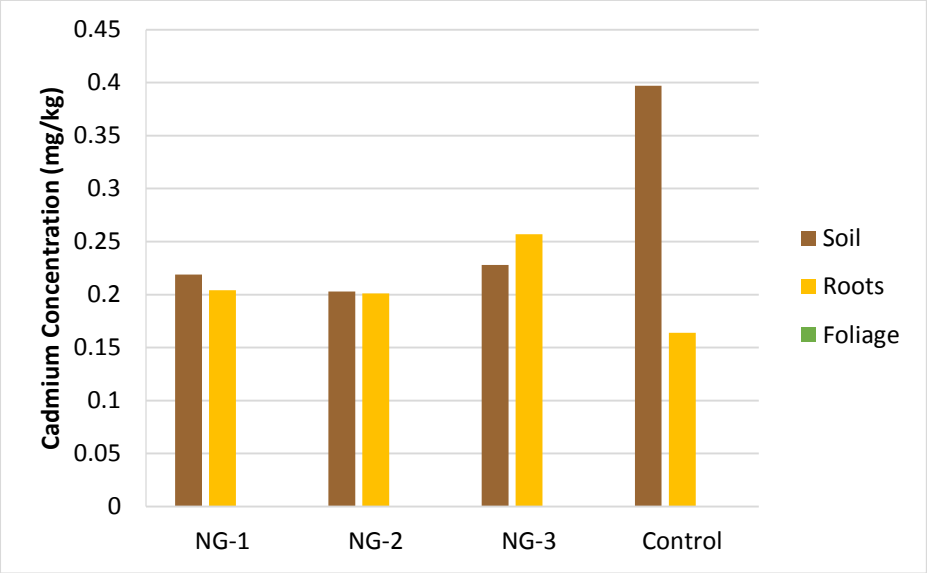


Figure 4.45. Cadmium concentrations for field samples of Purple Needlegrass

4.1.8. Lead Phytoextraction in the Field

The lead concentrations in the soil associated with the plant specimens collected were much lower than the action level of 49 mg/kg (Figures 4.46 to 4.54). At these low levels Purple Needlegrass showed the greatest plant tissue lead concentrations. Specimen NG-3 had a root cadmium concentration of 8.9 mg/kg and specimen NG-1 had a foliage cadmium concentration of 1.17 mg/kg (Figure 4.46) compared to a soil level of 17 mg/kg and 11 mg/kg respectively.

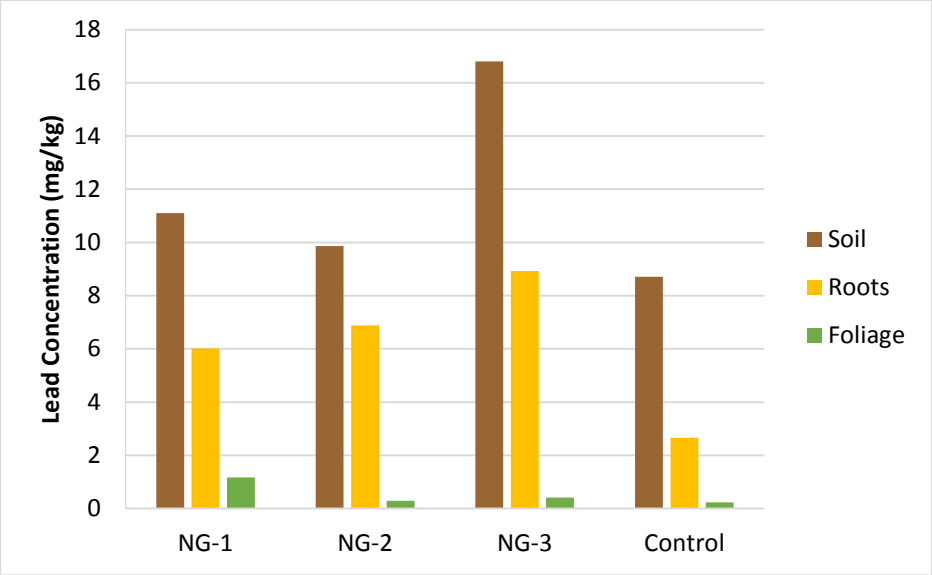


Figure 4.46. Lead concentrations for field samples of Purple Needlegrass

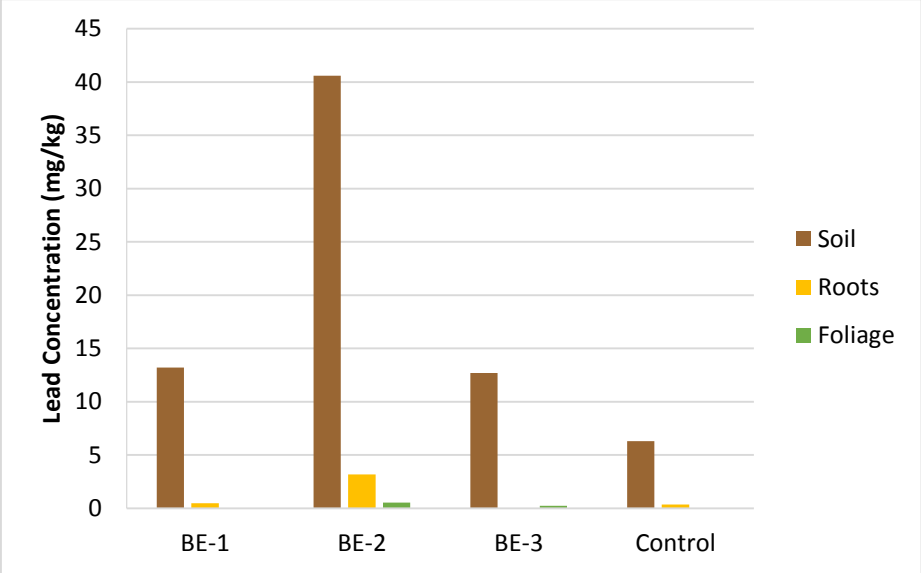


Figure 4.47. Lead concentrations for field samples of Blue Elderberry

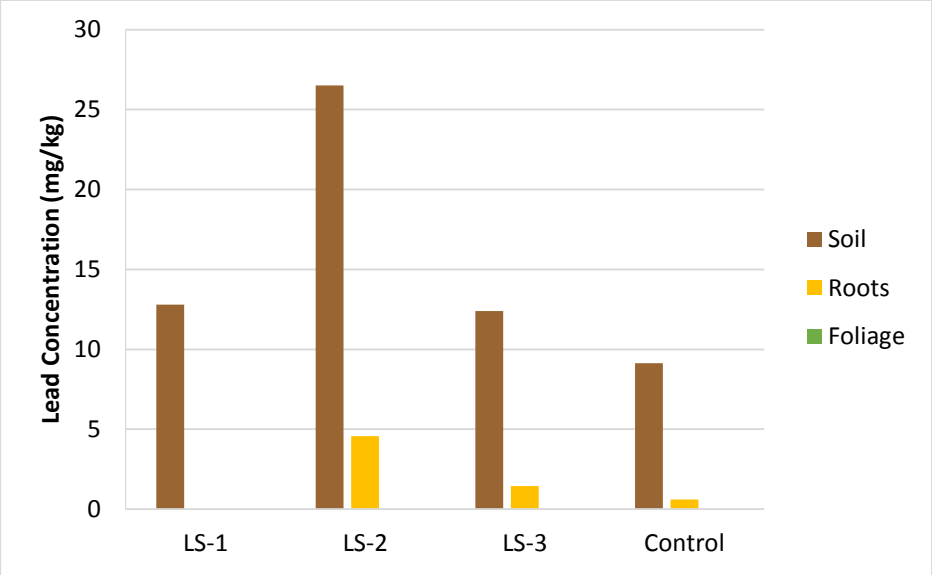


Figure 4.48. Lead concentrations for field samples of Laurel Sumac

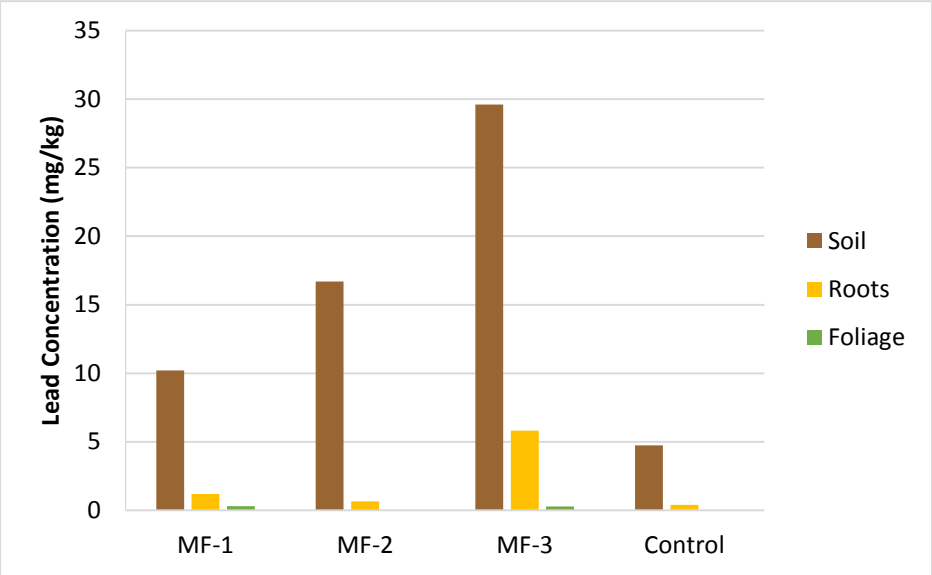


Figure 4.49. Lead concentrations for field samples of Mule Fat

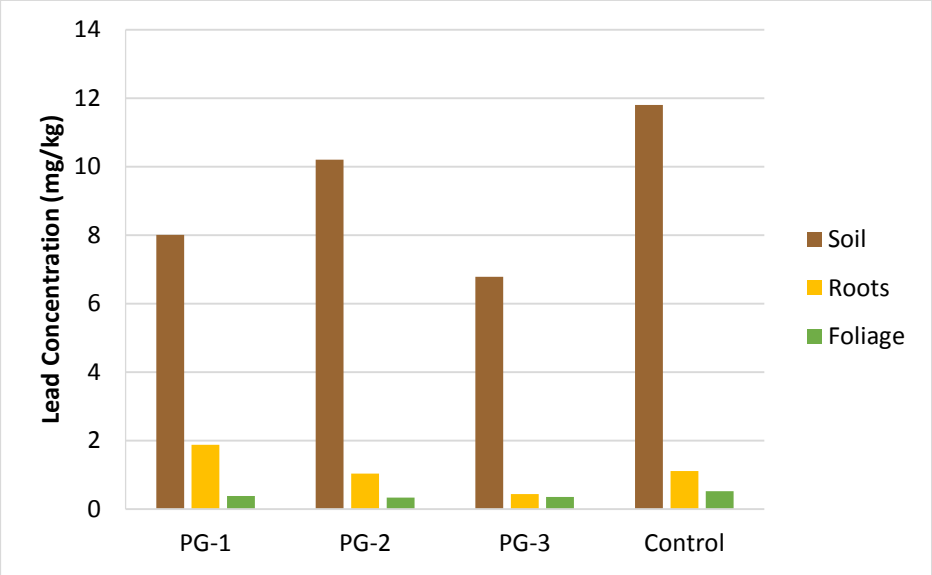


Figure 4.50. Lead concentrations for field samples of Palmer's Goldenbush

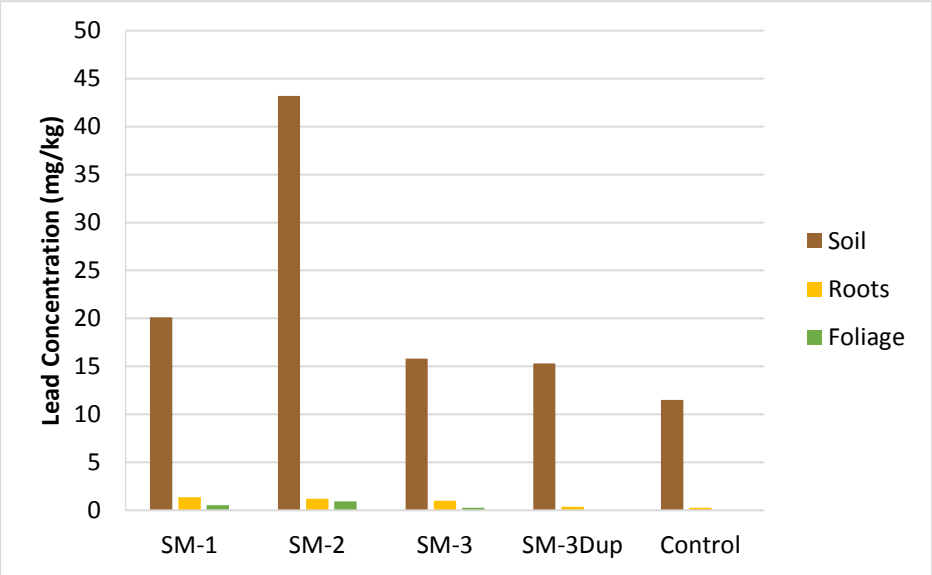


Figure 4.51. Lead concentrations for field samples of Summer Mustard

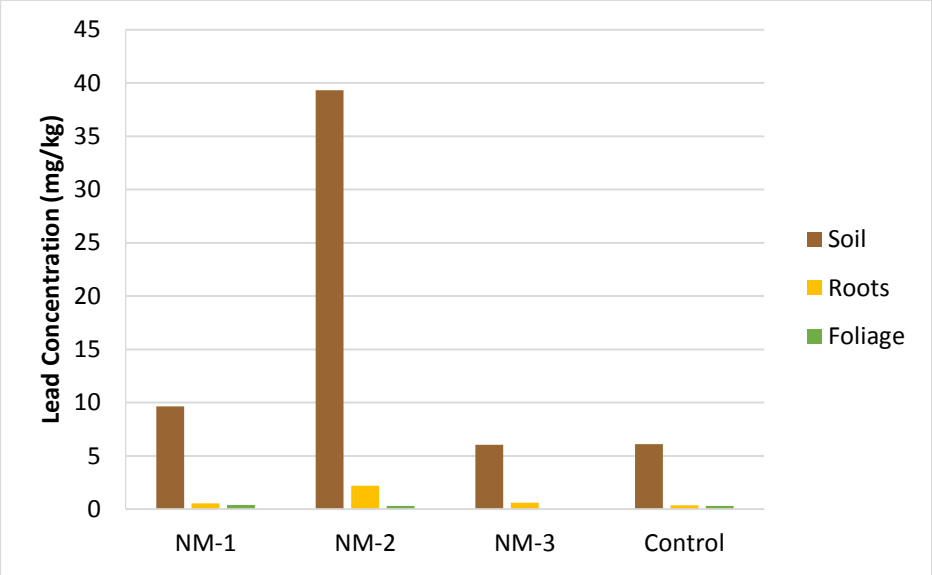


Figure 4.52. Lead concentrations for field samples of Narrowleaf Milkweed

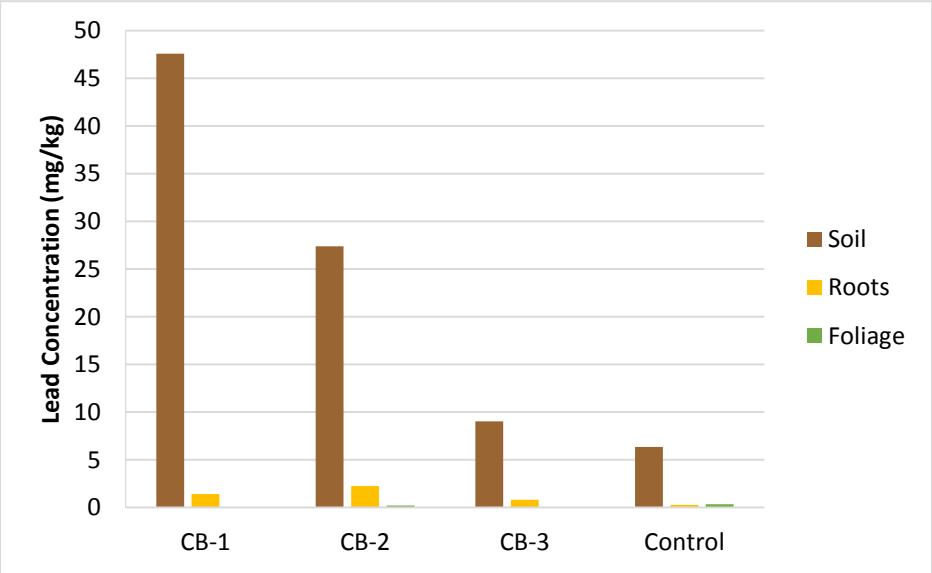


Figure 4.53. Lead concentrations for field samples of Coyote Brush

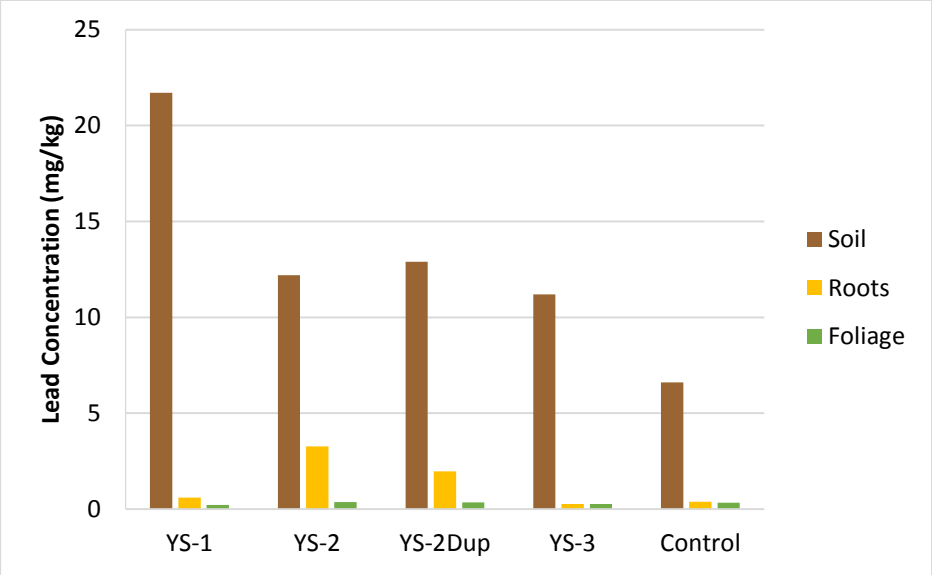


Figure 4.54. Lead concentrations for field samples of Yerba Santa

4.2. Phase II: Microcosm Results

The initial homogenized bulk soil was sampled at the beginning of the experiment. The microcosm soil was sampled again at 85 days after planting, and the soil, roots, and foliage were sampled at 211 days after planting. A summary of the microcosm sampling events is shown in Table 4.1. The sealed and sterilized unplanted microcosms were not sampled at 85 days to prevent any introduction of microbes into the sterile soil.

Results for each COI are described below in the following sections. In these sections, the concentrations of specific contaminants are summed within each category of contaminants. For example, all PAH compounds and all PCB congeners are summed, and the totals are reported in the figures in this section of the report. Concentrations of specific compounds/congeners are shown in additional graphs in Appendix A. Details of the statistical analysis results are shown in Appendix B.

4.2.1. Plant Growth in the Microcosms

All of the plants grown for the greenhouse experiments were healthy (Figure 4.55 A) with the exception of the Coyote Brush grown in soil with added chelating agent (EDTA). The plants in this treatment exhibited lower biomass production, reduced water uptake, and more dead leaves and stems than the Coyote Brush in other treatments (Figure 4.55 B).

Table 4.1. Summary of microcosm sampling events

	Number of Replicates Sampled					
	0 Days		85 Days		211 Days	
Microcosm	**Soil**	**Roots/Foliage**	**Soil**	**Roots/Foliage**	**Soil**	**Roots/Foliage**
Coyote Brush (PA)	15	-	5	-	5	5
Mule Fat (PB)	15	-	5	-	5	5
Purple Needlegrass (PC)	15	-	5	-	2	2
Fertilized Coyote Brush (FPA)	15	-	5	-	5	5
Chelated Coyote Brush (CPA)	15	-	5	-	3	3
Sterilized Purple Needlegrass (SP)	15	-	5	-	2	2
Unplanted (U)	15	-	5	-	5	5
Sterilized Unplanted (SU)	15	-	-	-	5	5

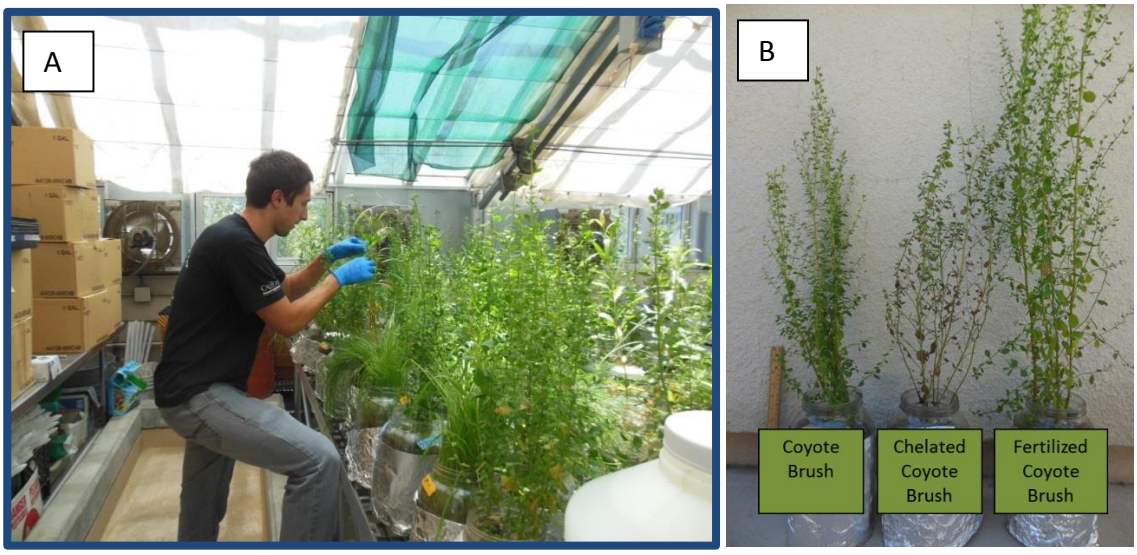


Figure 4.55. A) Graduate student Matthew Poltorak tending to greenhouse plants, B) Untreated, chelated, and fertilized Coyote Brush plants

4.2.2. Petroleum Hydrocarbons in Microcosms

EFH concentrations were not measured for the microcosm plant tissue since the field screening showed that plant-produced compounds completely masked any petroleum hydrocarbons that might have been taken into the plant. Total EFH concentrations in the soil of each microcosm are shown in Figure 4.56 for the duration of the experiment. No significant change in soil EFH concentrations were observed for any of the microcosms during the first 85 days. All of the treatments showed an apparent increase in EFH soil concentrations at Day 211 of the experiment. However, the 211-day samples were analyzed by a different analytical lab than the 0 and 85-day samples (initial and midpoint EFH analyses were performed by EMAX labs while the final analysis on Day 211 was performed by Lancaster labs). The same anomaly was observed in a companion study on bioremediation, and for that study the final samples were resent to the original laboratory, which then reported EFH concentrations similar to those reported for Days 0 and 85. Therefore the 211-day EFH data for this phytoremediation study are not considered reliable and are not reported here. Statistical analysis of the final EFH soil concentrations indicates that none of the treatments had any significant effect except that the sterilized unplanted control had a lower total EFH concentration than the fertilized Coyote Brush treatment (Appendix B). These results indicate that the plants did not enhance the degradation of petroleum hydrocarbons. Also, since the final EFH concentration in the unplanted soil was not lower than that of the sterilized control biodegradation of EFH in the soil was probably also minimal.

An important consideration is that the EFH concentrations measured in this study may include natural organic material (NOM). GC/MS analysis of the site soils indicates that these soils contain natural organic acids, such as palmitic acid, and such NOM elutes on the GC in the same range as petroleum hydrocarbons. Therefore the reported EFH concentrations can be greater than the actual concentration of petroleum-based compounds. Quantification of the NOM constituents in the EFH measurements of site soils is currently being pursued by the Cal Poly researchers.

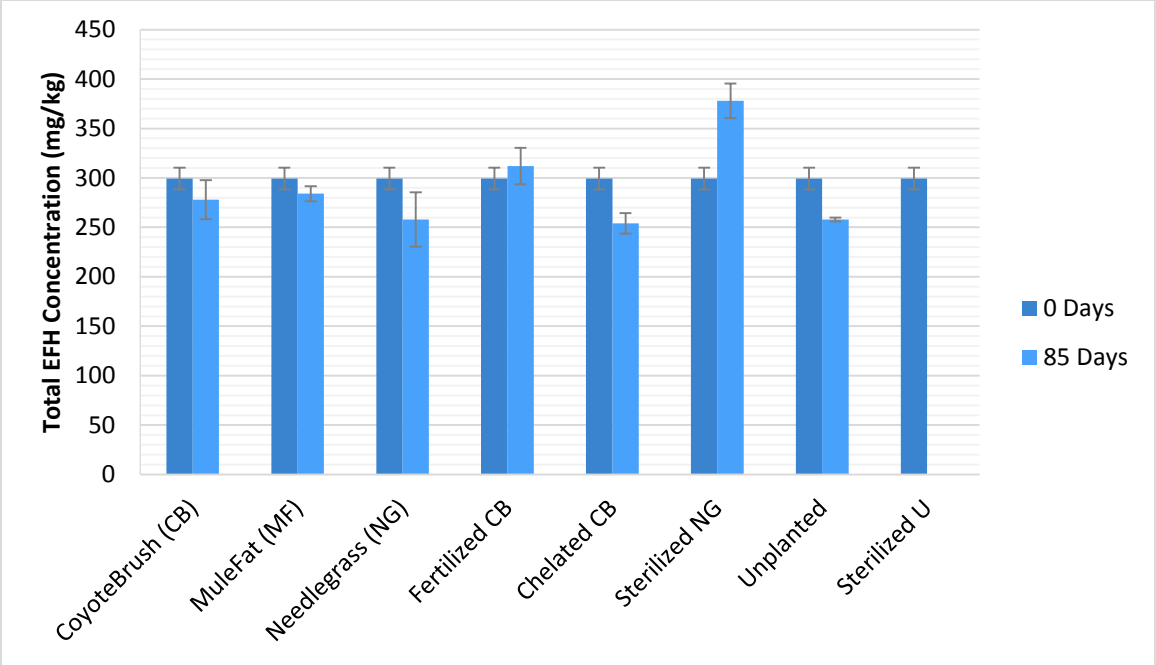


Figure 4.56. Total EFH concentrations in microcosm soil after 0, 85, and 211 days (error bars denote standard error of the mean)

4.2.3. Polyaromatic Hydrocarbons in Microcosms

Total PAH concentrations in the soil of each microcosm treatment decreased from 0 to 85 days (Figure 4.57). However, all of the total PAH concentrations appeared to increase by Day 211 to concentrations greater than the initial concentrations except in the sterilized unplanted control. These increases could be a laboratory anomaly similar to the EFH analysis. An analysis of variance between the treatments at 211 days showed that none of the total PAH concentrations were statistically different at the 95% confidence interval ($p = 0.094$). An analysis of variance between the treatments at 85 days also showed that none of the total PAH concentrations were statistically different at the 95% confidence interval ($p = 0.868$). Since the soil PAH concentrations in the unplanted control were not lower than those of the sterilized control, biodegradation of PAHs in the soil was probably minimal.

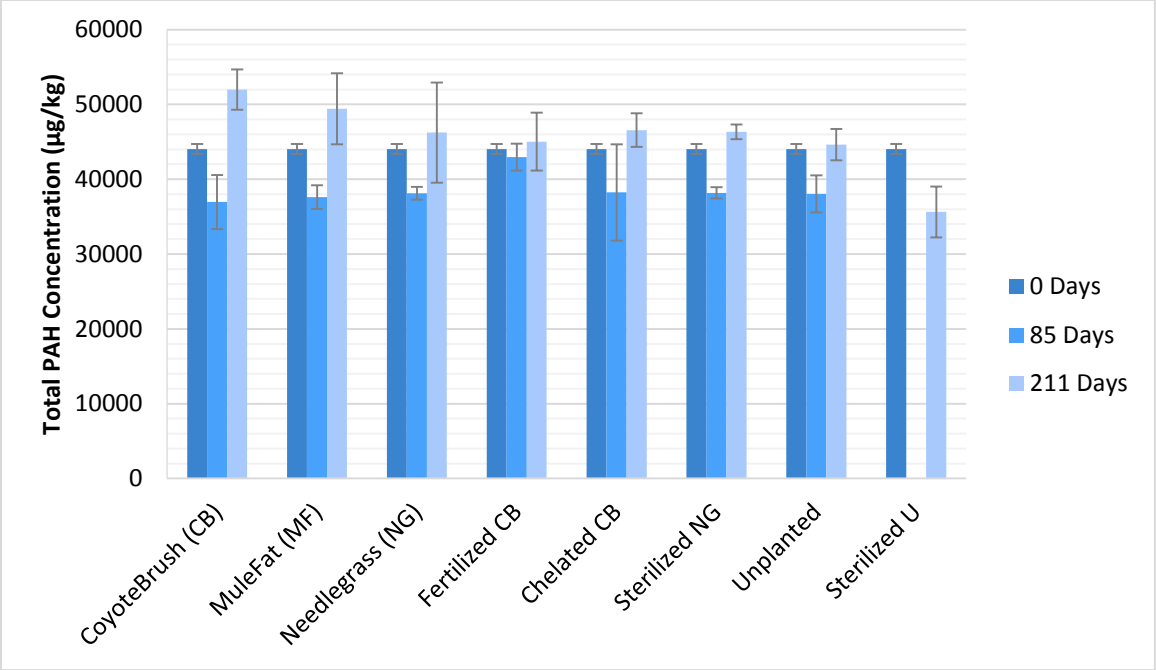


Figure 4.57. Total PAH concentrations in microcosm soil after 0, 85, and 211 days

All of the plants showed some uptake of PAHs into the roots (Figure 4.58). Sterilized Purple Needlegrass (SP) and Purple Needlegrass (PC) showed the highest average PAH concentrations in the root tissue out of all the treatments. The root uptake for Purple Needlegrass grown in sterilized and unsterilized soil are not statistically different ($p = 0.358$, 2-sample t-test, 95% confidence), indicating the root uptake by Purple Needlegrass was not affected by soil microbes. Purple Needlegrass in the microcosms did not show any uptake into the foliage. Coyote Brush, chelated Coyote Brush, fertilized Coyote Brush, and Mule Fat showed very slight uptake of PAHs into the foliage. Despite observed uptake of PAHs into roots and foliage, this uptake did not significantly reduce the soil concentrations during this time frame, as described above.

In the field screening, Mule Fat specimens showed PAH concentrations greater than 4,000 µg/kg in their foliage and no PAHs in their roots. This measure of PAH was attributed to production of phytogenic compounds by Mule Fat as described above. In contrast, in the microcosms the average foliage concentration was only 209 µg/kg while the average root concentration was 6,500 µg/kg. The Mule Fat in the microcosms may have been too young to produce the phytogenic compounds that were observed in the field screening, or perhaps field conditions influenced the production of these compounds. Regardless of the production of phytogenic compounds observed in the field, Mule Fat does appear to phytoextract PAHs from the soil based on soil and root PAH concentrations (Figure 4.58).

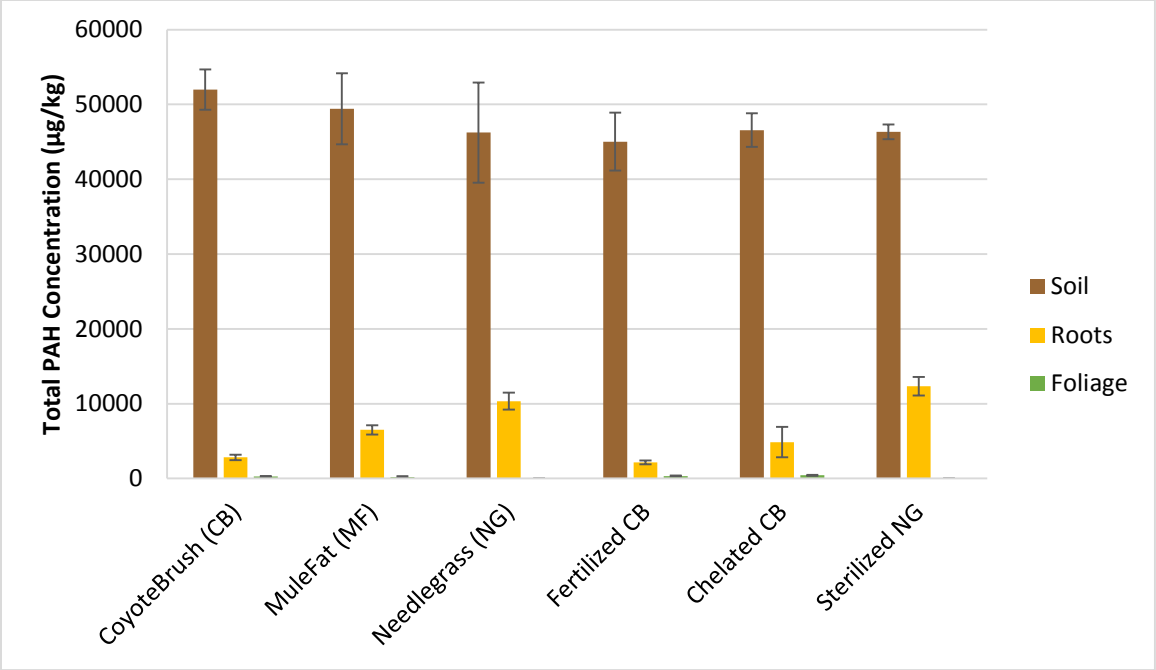


Figure 4.58. Total PAH concentrations in soil, roots, and foliage across treatments

4.2.4. Polychlorinated Biphenyls in Microcosms

All of the microcosm treatments, including the unplanted, sterile control, showed decreases in soil PCB concentrations (measured as Aroclor 1260) relative to the initial soil concentration (Figure 4.59). The soils planted with Purple Needlegrass and chelated Coyote Brush exhibited the greatest decreases in soil PCB concentration (49% for Purple Needlegrass and 51.4% for chelated Coyote Brush, compared to 37% for the sterilized unplanted soil). However, these differences were not statistically significant at the 95% confidence level.

Given the decrease in PCB concentrations observed for the sterile controls, it is likely that abiotic processes contributed to the observed decreases in soil PCB concentrations in the microcosms. Researchers have reported significant losses of PCBs due to adsorption to glass from aqueous solutions (Lung et al. 2000), and thus it is likely that such adsorption may have occurred from the moist soil samples used in the phytoremediation microcosms.

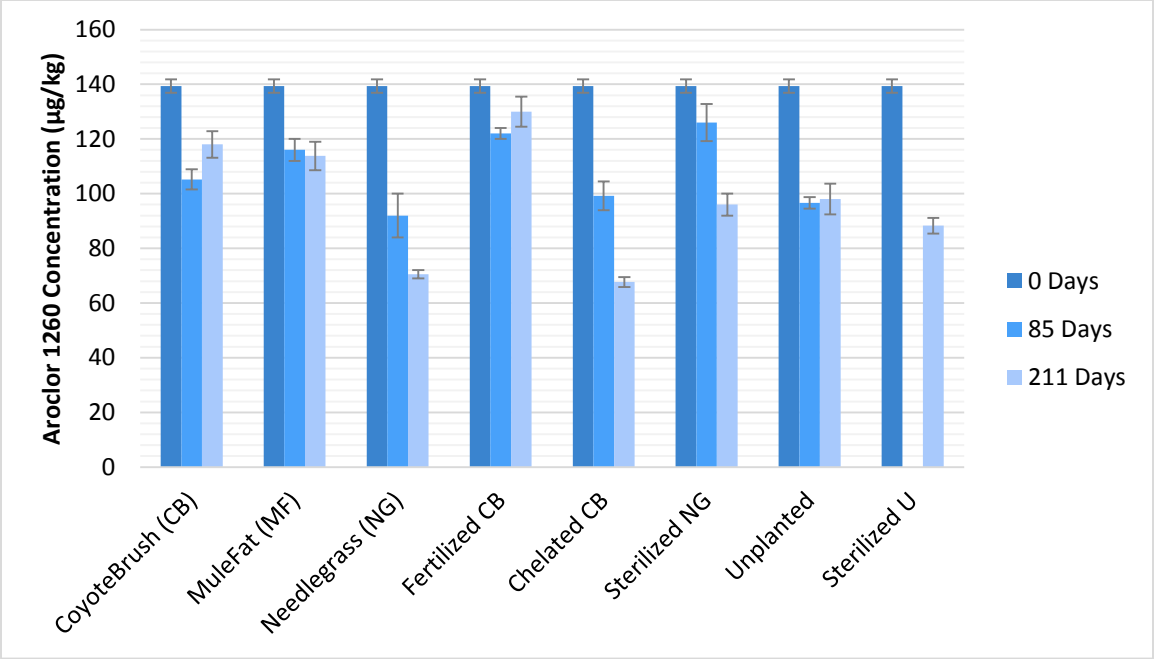


Figure 4.59. Aroclor 1260 concentrations in microcosm soil after 0, 85, and 211 days

During the field screening, none of the Purple Needlegrass specimens had PCBs in the associated soil, thus it was especially important to test the plant tissue for PCBs in the final microcosm sampling event. None of the other species sampled in the field showed uptake of PCBs into their tissue. The microcosm analysis showed no PCBs in the roots or foliage of Purple Needlegrass after 211 days, indicating that Purple Needlegrass did not phytoextract PCBs from the soil. Roots and foliage of Coyote Brush and Mule Fat were not tested for PCBs in the microcosm experiment since previous field screening results showed that these species did not phytoextract PCBs. Thus the PCB reductions seen in soil planted with Purple Needlegrass, chelated Coyote Brush, and sterilized Purple Needlegrass would not be expected to be a result of phytoextraction.

4.2.5. Chlorinated Dioxins/Furans in Microcosms

Soil in microcosms planted with Coyote Brush with and without fertilizer showed significant decreases in chlorinated dioxin/furan concentrations compared to the unplanted and sterilized controls (Figure 4.60). Some of the changes observed could be attributed to the natural variability of contaminant concentrations in the soil. For example, while most of the sampling replicates from the initial soil were around 28,000 ng/kg, one individual replicate had a total dioxin/furan concentration of over 43,000 ng/kg. It is possible that there were concentrated clusters of dioxins/furans that increased the concentration of a particular replicate as described for PCBs above.

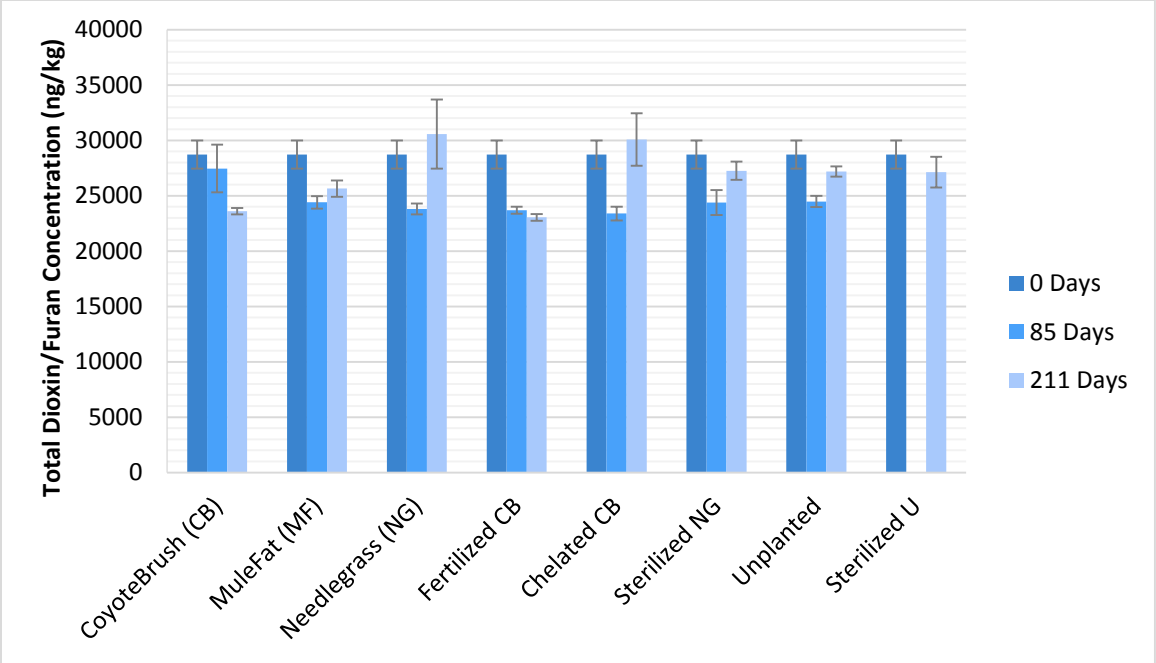


Figure 4.60. Total chlorinated dioxin/furan concentrations in microcosm soil after 0, 85, and 211 days

Purple Needlegrass growing in either sterilized or unsterilized soils showed the highest average chlorinated dioxin/furan concentrations in the root tissue out of all the plants (Figure 4.61). Purple Needlegrass also showed the greatest uptake of dioxins/furans into the foliage, although much less than that observed in the roots (Figure 4.61). The dioxin congener with the highest concentration in the soil was OCDD and this congener was also was the highest in the Purple Needlegrass roots (Appendix A). Soils planted with Coyote Brush with and without fertilizer exhibited the greatest significant ($p = 0.036$, $p = 0.022$) decrease of dioxins/furans concentrations (17.8% and 19.8% respectively).

In the case of Purple Needlegrass, the observed root uptake (Figure 4.61) did not appear to contribute to significant reductions in soil dioxin concentrations (Figure 4.60). However, for Coyote Brush the observed uptake of dioxins (Figure 4.61) corresponded with a decrease in soil concentrations of dioxins (Figure 4.60). Thus, plant uptake could potentially be a mechanism of remediation in the case of Coyote Brush. The limited effect of Purple Needlegrass may have been due to low biomass growth. The fertilized plants showed the most biomass production and vigor and the most rapid reduction in soil dioxin/furan concentrations. Graphs of individual dioxin/furan congener concentrations that were significantly high in the soil are shown in Appendix A.

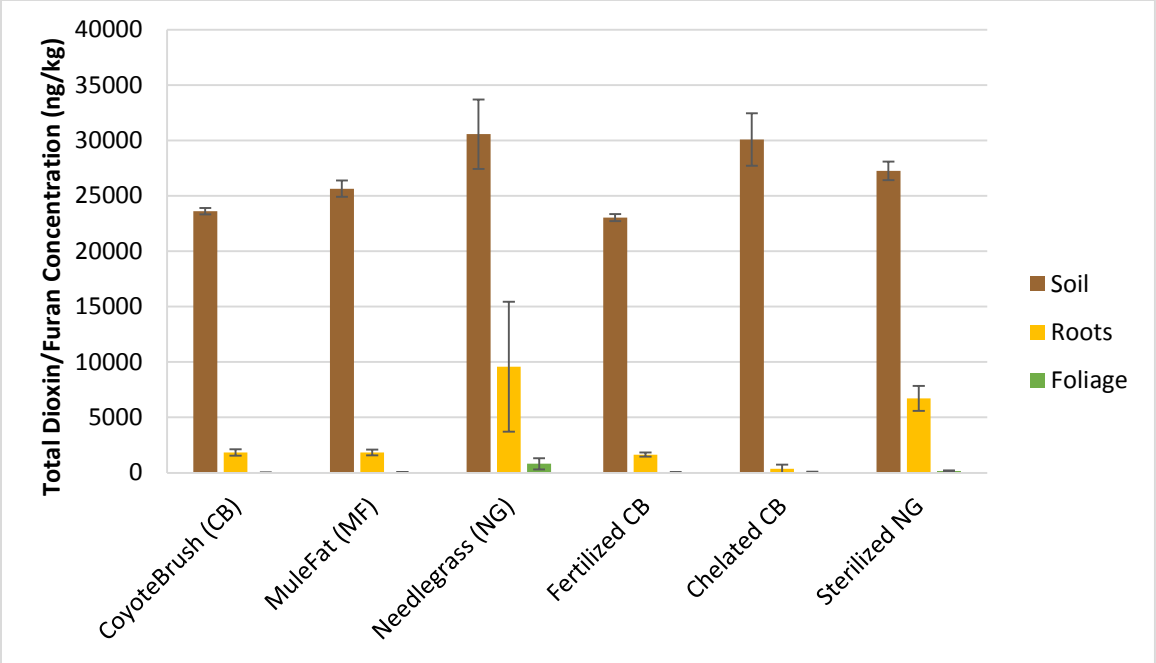


Figure 4.61. Total dioxin/furan concentrations in soil, roots, and foliage

4.2.6. Mercury

All of the treatments, including the sterilized unplanted microcosms, showed a slight decrease in mercury soil concentrations (Figure 4.62). No statistical differences were observed bewteen final mercury soil concentrations between treatments ($p = 0.110$, 95% confidence). Since the sterilized, unplanted control showed a similar decrease in mercury soil concentration as the planted microcosms, these reductions are not likely to be the result of phytoremediation.

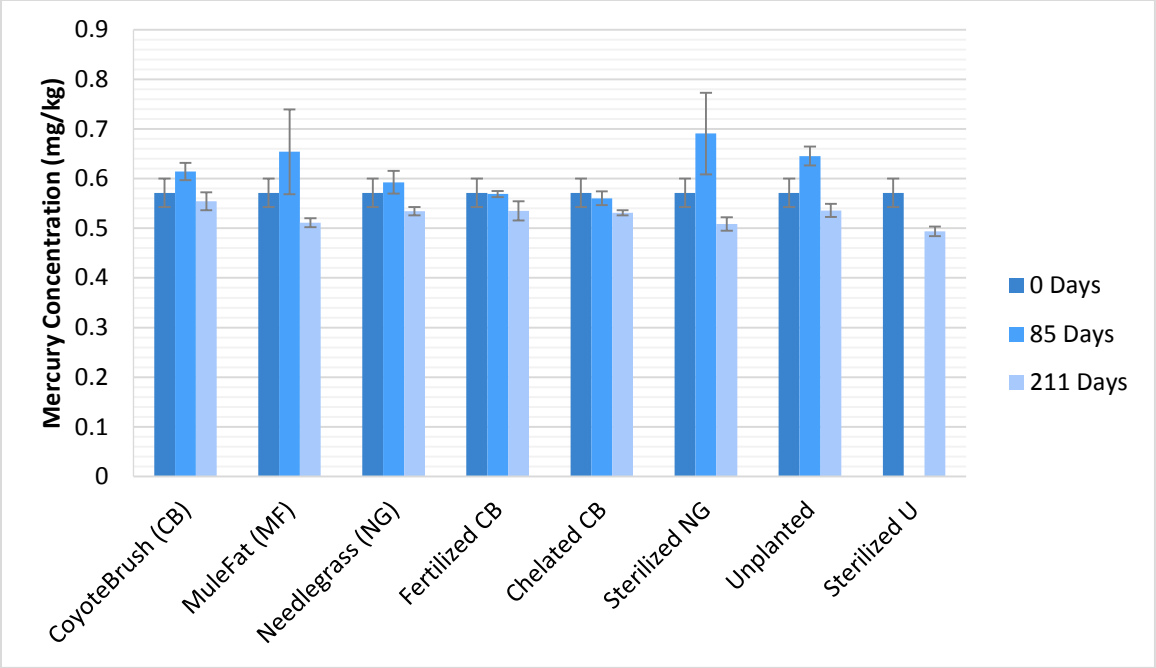


Figure 4.62. Mercury concentrations in microcosm soil after 0, 85, and 211 days

Mercury was detected in the roots of Mule Fat, fertilized Coyote Brush, and Purple Needlegrass growing in sterilized or unsterilized soil (Figure 4.63). Mercury was also detected in the foliage of Purple Needlegrass with and without sterilized soil. However, it was difficult to precisely separate the roots from the foliage for Purple Needlegrass. Since the concentration in the roots and foliage was below 0.1 mg/kg in most cases, and since all of the treatments have similar soil concentrations at Day 211, it is unlikely that mercury uptake was significant for any of the treatments in this study.

The EDTA chelating amendment did not enhance uptake of mercury into Coyote Brush roots or foliage. In fact there was less mercury uptake observed for Coyote Brush with EDTA than for fertilized Coyote Brush without EDTA (Figure 4.63).

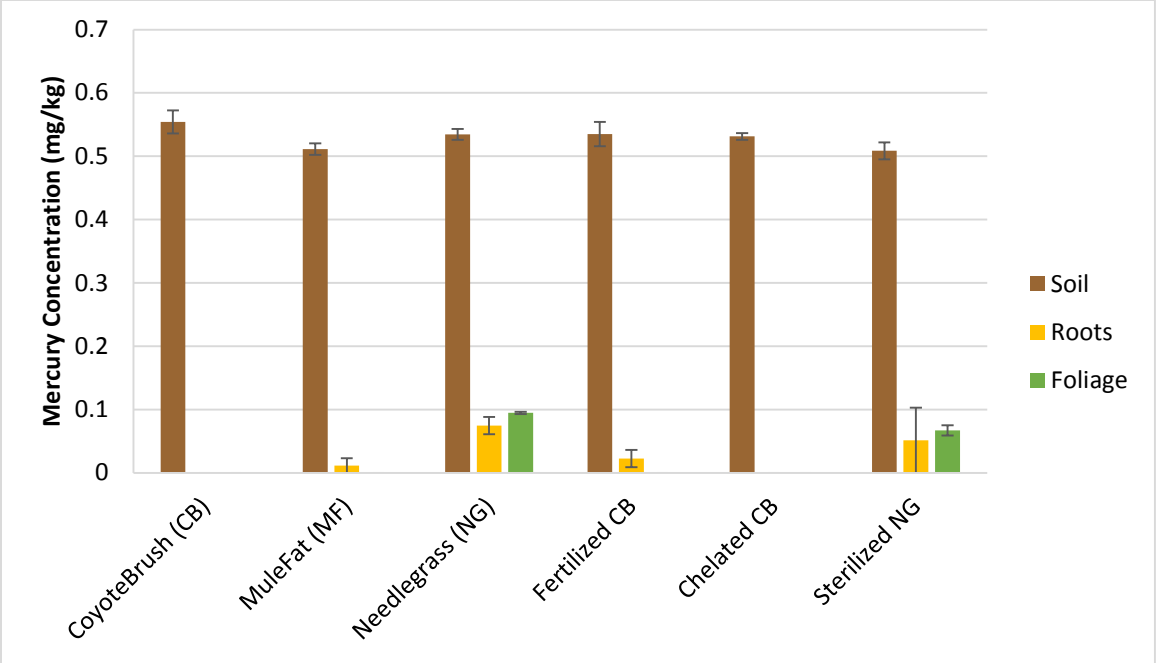


Figure 4.63. Mercury concentrations in soil, roots, and foliage across treatments

4.2.7. Silver

All of the treatments at 211 days showed higher soil concentrations for silver than the initial bulk soil (Figure 4.64). Silver was observed in the root tissue of all plant species tested (Figure 4.65). The Purple Needlegrass treatment showed statistically greater concentrations of silver in the roots compared to the other treatments ($p < 0.05$, one way ANOVA). Purple Needlegrass growing in sterilized and unsterilized soil was the only plant that showed uptake into the foliage. However, this apparent uptake of silver was minimal and could be attributed to the difficulty in distinguishing/separating the roots from the foliage for grass samples. EDTA did not enhance silver uptake into the Coyote Brush roots since the root concentrations for Coyote Brush and the chelated Coyote Brush were not statistically different ($p = 0.553$).

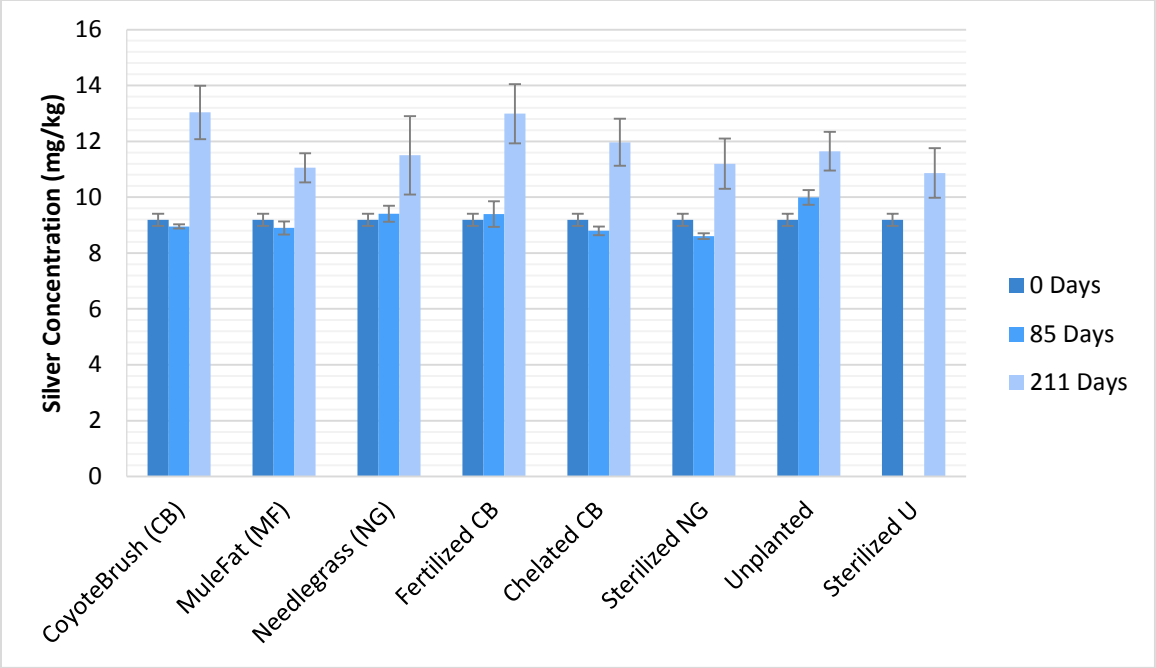


Figure 4.64. Silver concentrations in microcosm soil after 0, 85, and 211 days

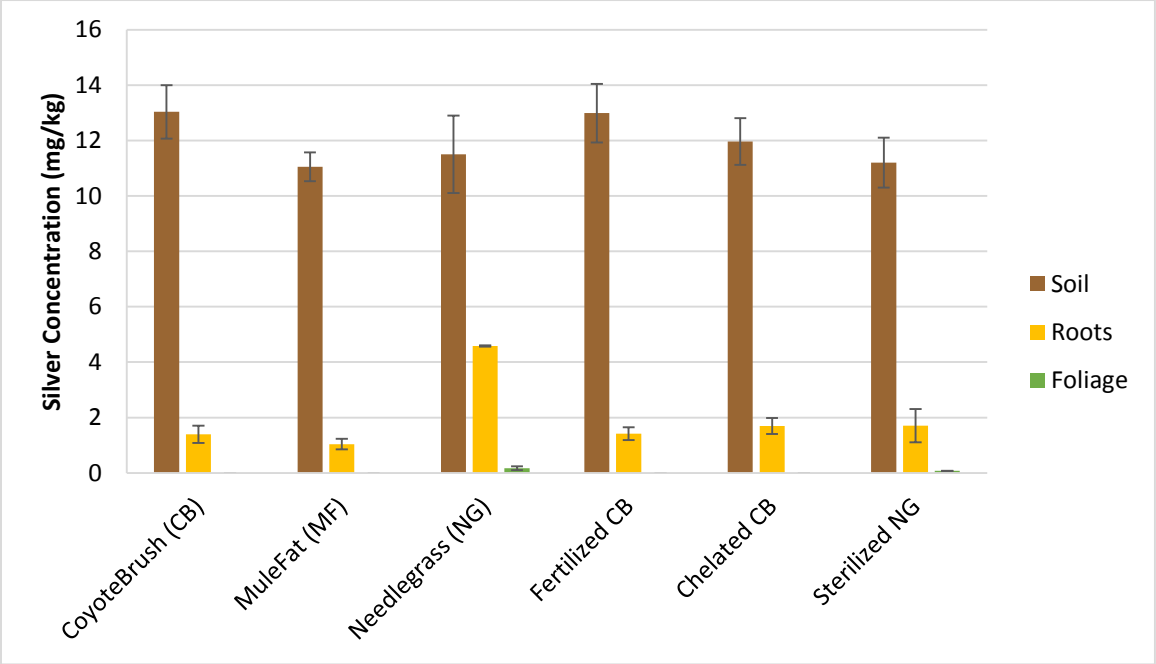


Figure 4.65. Silver concentrations in soil, roots, and foliage across treatments

4.2.8. Cadmium

Cadmium concentrations in microcosm soils were similar for all treatments, indicating that none of the plant species significantly enhanced the removal of cadmium from the soil (Figure 4.66). All of the treatments showed an average root concentration of cadmium that was similar to or greater than the soil concentration (Figure 4.67) which was only slightly above the action level of 0.7 mg/kg. All of the plants also showed uptake into the foliage (Figure 4.67). The addition of the EDTA did not increase the uptake of cadmium into the roots of Coyote Brush since the root concentrations were not statistically different ($p = 0.207$) with and without EDTA.

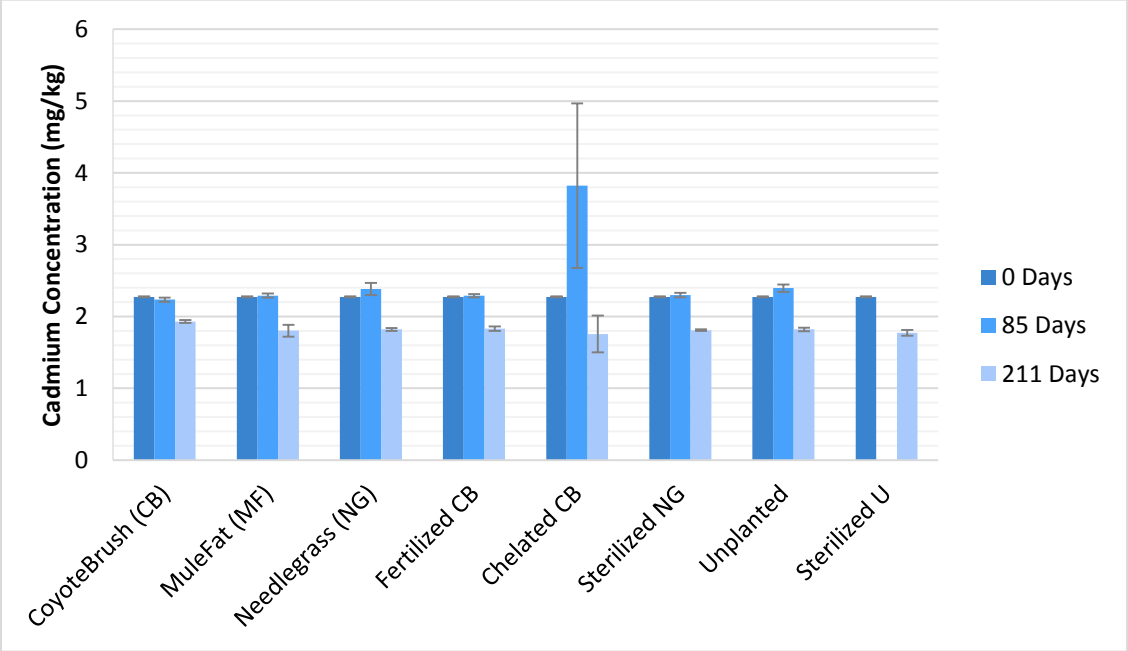


Figure 4.66. Cadmium concentrations in microcosm soil after 0, 85, and 211 days

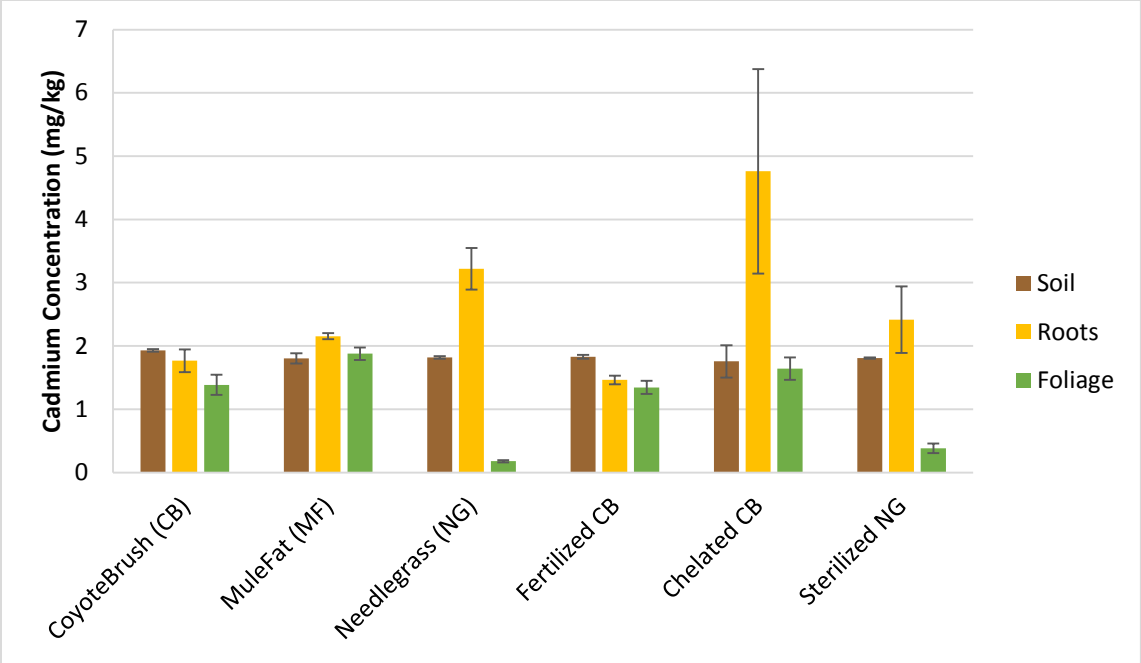


Figure 4.67. Cadmium concentrations in soil, roots, and foliage across treatments

4.2.9. Lead

Lead concentrations in the soils were unchanged over 211 days and were not statistically different (Figure 4.68). The plants clearly did not contribute to any significant lead concentration reductions in the soil. Although no changes in soil concentrations were observed, lead was detected in the roots and foliage of some plants (Figure 4.69). The EDTA enhanced the phytoextraction of lead by Coyote Brush, with root concentrations increasing from 6.53 mg/kg to 42.20 mg/kg with EDTA (95% confidence; $p = 0.016$). EDTA also enhanced the translocation with an increase from 3.7 mg/kg to 5.8 mg/kg in the foliage concentration with EDTA (95% confidence; $p < 0.05$). This increase of lead uptake by the chelated specimens may have caused the physiological damage that was observed for this treatment (Figure 4.55). Lead accumulation in plant tissue has been shown to impair various morphological, physiological, and biochemical functions in plants, either directly or indirectly (Pourrut et al. 2011).

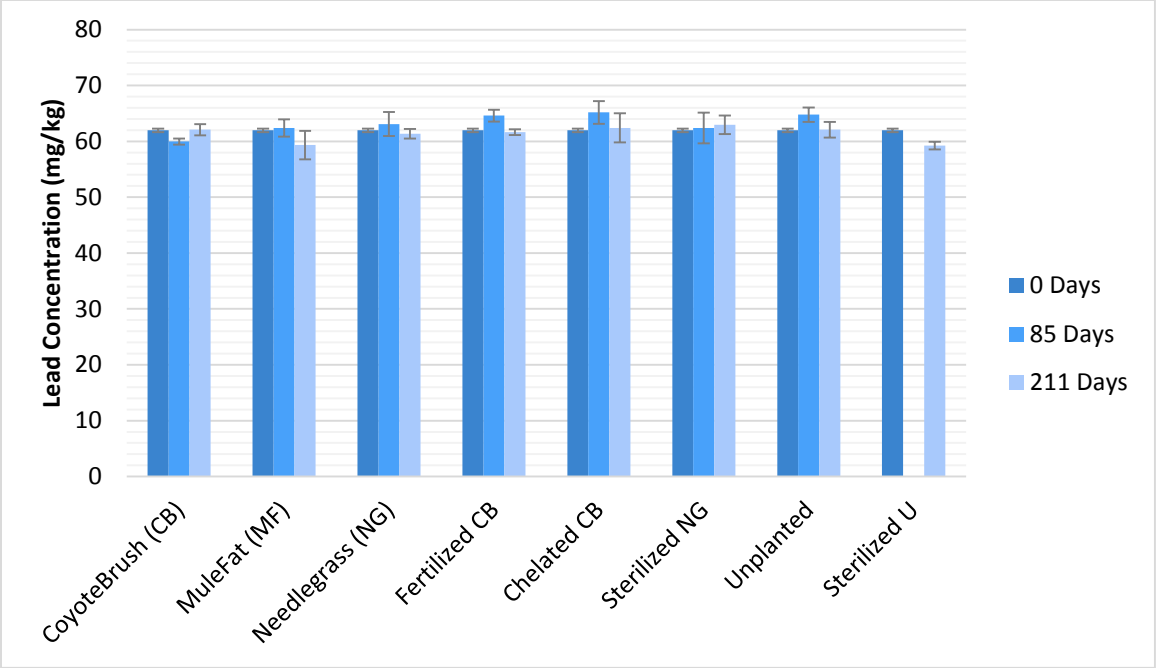


Figure 4.68. Lead concentrations in microcosm soil after 0, 85, and 211 days

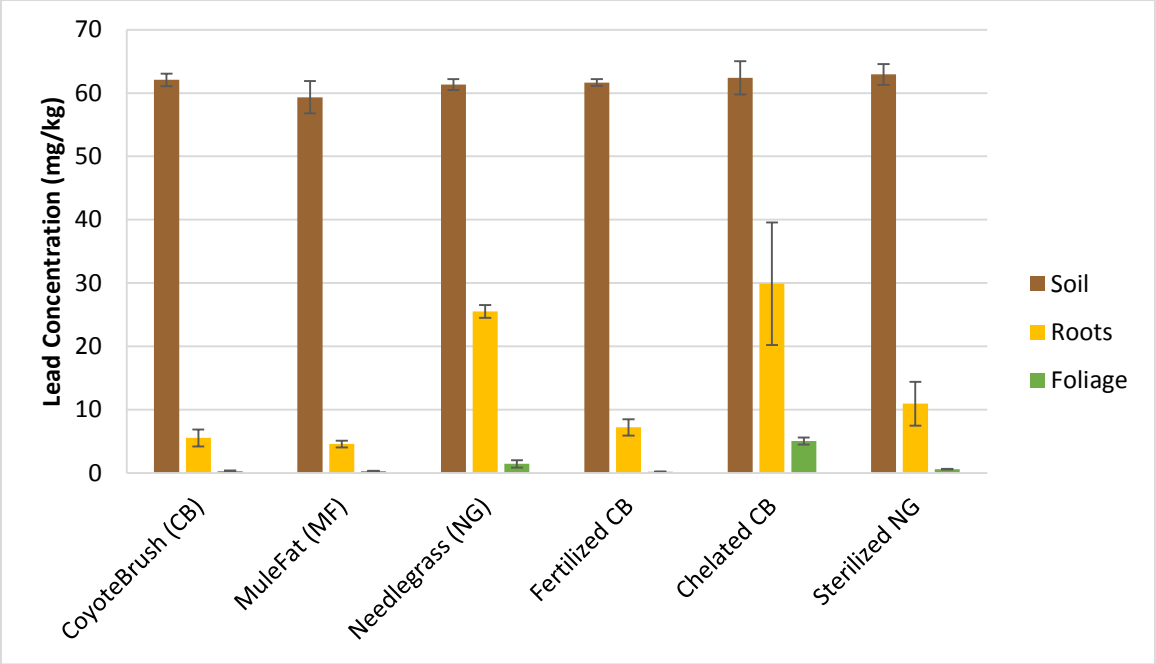


Figure 4.69. Lead concentrations in soil, roots, and foliage across treatments

4.2.10. Phytovolatilization

The off gas from plants growing in contaminated soil was analyzed by GC/MS, after trapping in carbon sorbent tubes. Several chemicals were identified in this off-gas, all of which appear to be produced by plants. The most commonly detected compounds were d-limonene, stearic and oleic acids, and stigmastan-3, 5-diene. Limonene is an essential oil produced by some plants of the *Baccharis* genus, with d-limonene being the more commonly found enantiomer in plants (Concha et al. 2014). Stearic and oleic acids are fatty acids that are also produced by plants of the *Baccharis* genus and also found on human skin (Chang et al. 2008). Stigmastan-3, 5-diene is an antimicrobial compound emitted from avocado roots (Sánchez-Pérez et al. 2009) and appears to be produced by the greenhouse plants. These compounds were detected in the ambient greenhouse air and the air from the bags enclosing the planted microcosms (Figure 4.70). However, these compounds were not detected from the bags enclosing an empty jar or unplanted soil, thus it can be concluded that the plants in the greenhouse produced the detected chemicals.

None of the COIs were detected in the GC/MS analysis of the plant off-gas. Mercury volatilization was not tested for. Other air sampling methods use alternative sorbents for adsorbing PCBs and chlorinated dioxins/furans (Centers for Disease Control and Prevention 2014).

4.2.11. Soil Microbial Characterization Using Terminal Restriction Fragment Analysis

Terminal restriction fragment (TRF) analyses were performed on soils collected from the microcosms to determine the effects of the plants on the soil microbial communities. The four types of microcosms tested were those planted with Purple Needlegrass (in both sterilized and unsterilized soil), unplanted, and sterilized unplanted microcosms. Multi-dimensional scaling (MDS) analysis of the TRF data indicates that the microbial populations in the sterilized, unplanted soil are grouped very separately from the other treatments (Figure 4.71). This is expected because the sterilization process likely killed most bacterial and fungal species. MDS analysis of the unsterilized soils also indicates differences between the three treatments (Figure 4.72). The sterilized Purple Needlegrass is grouped together separate from the other treatments with the Purple Needlegrass located somewhere between the unplanted and sterilized Purple Needlegrass treatments. The unplanted control is just over 60% similar to the Purple Needlegrass (Figure 4.73), which indicates that the Purple Needlegrass may have stimulated the rhizosphere and altered the soil microbial community. However, an analysis of similarities (ANOSIM) indicates that the variability in the data did not allow for significance between the Purple Needlegrass and unplanted control because there were too few replicates (Appendix B).

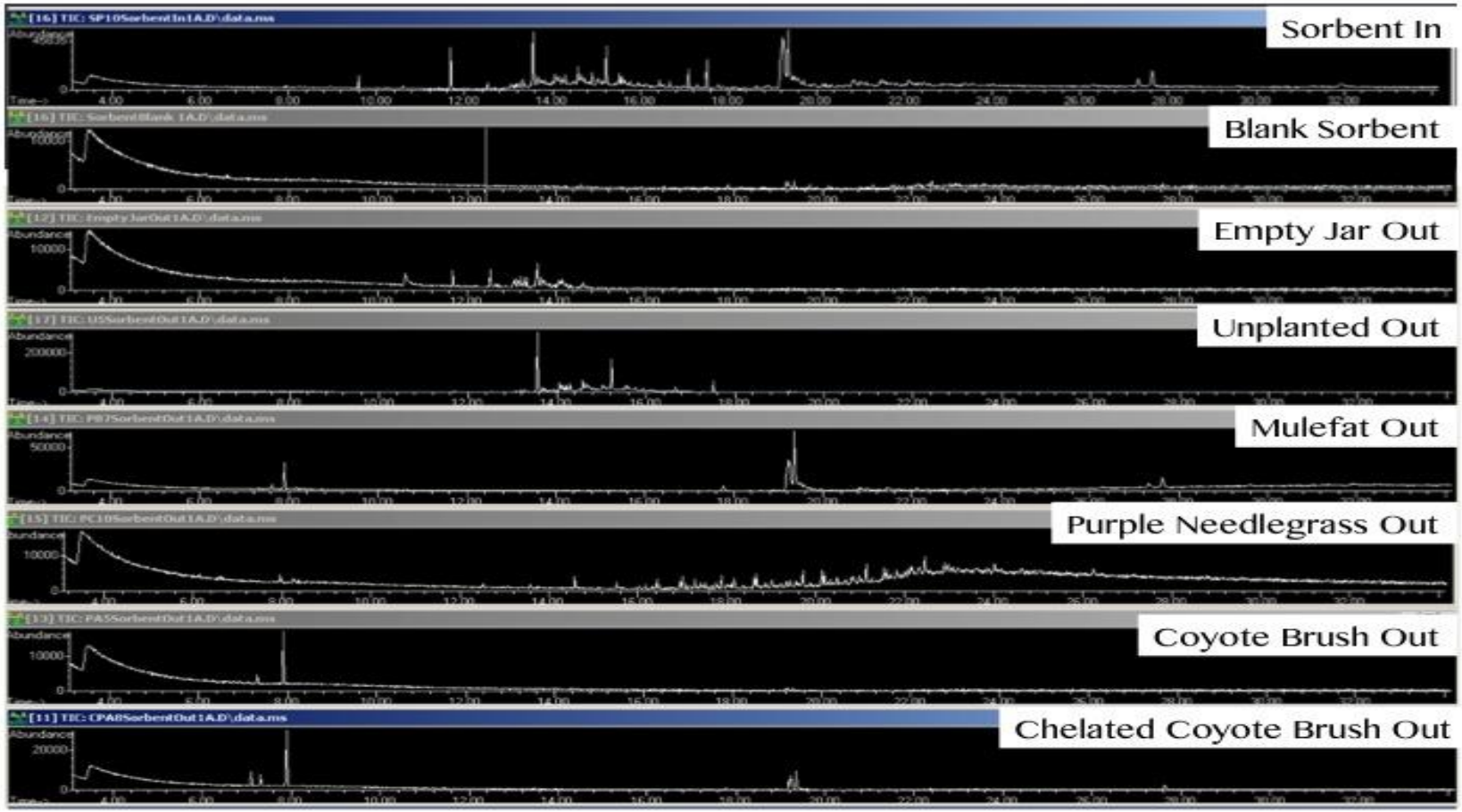


Figure 4.70. Gas chromatography peaks from volatilization sampling

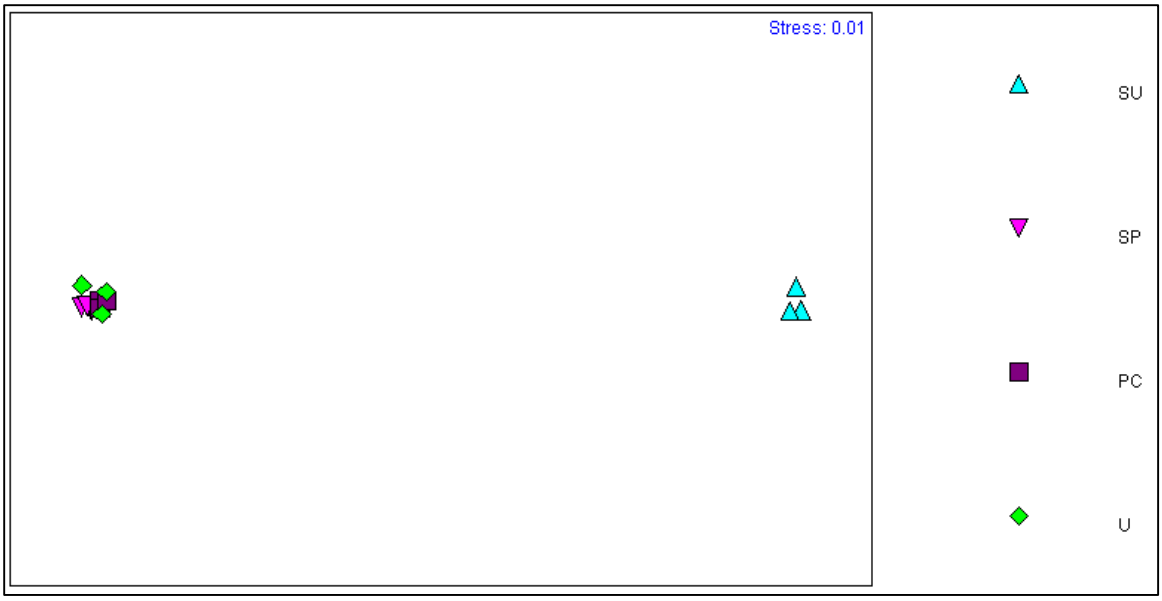


Figure 4.71. Microbial population groupings (MDS) as determined by TRF analysis of Purple Needlegrass (PC), sterilized Purple Needlegrass (SP), unplanted (U), and sterilized unplanted (SU) treatments

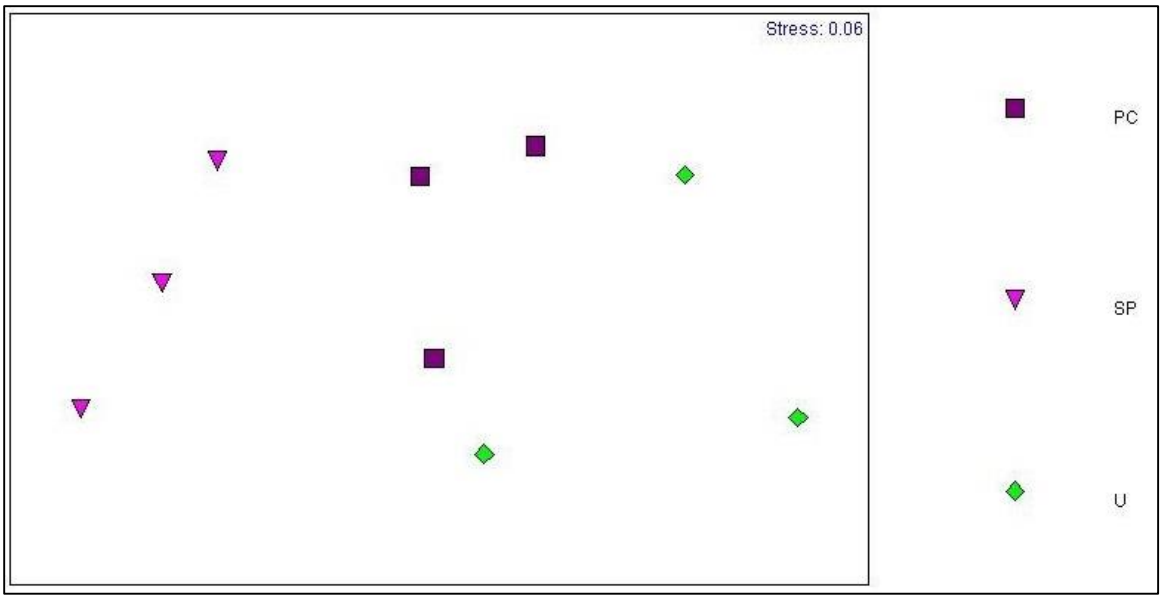


Figure 4.72. Microbial population groupings (MDS) as determined by TRF analysis of Purple Needlegrass (PC), sterilized Purple Needlegrass (SP), and unplanted (U) treatments

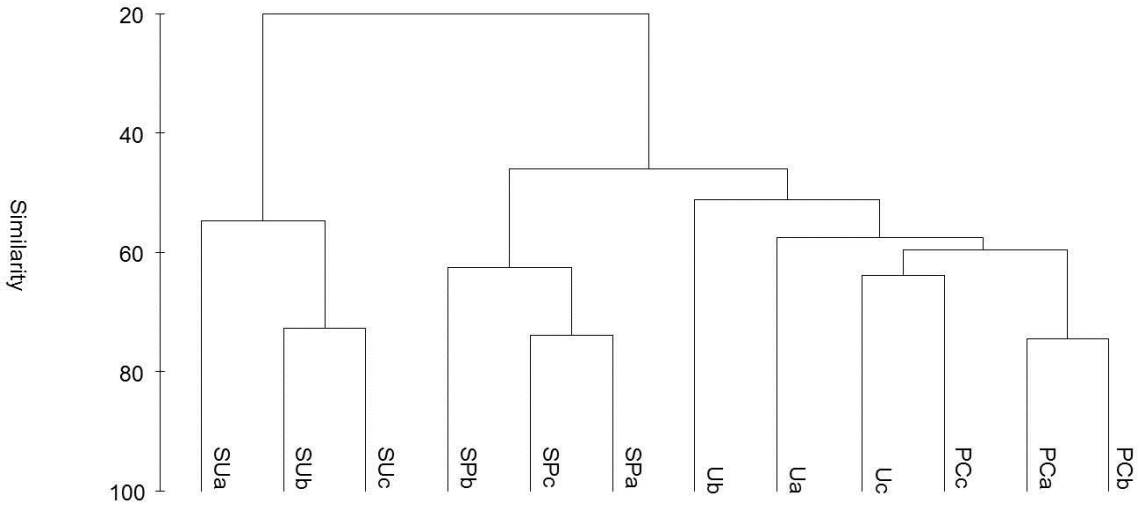


Figure 4.73. Dendogram for Purple Needlegrass (PC), sterilized Purple Needlegrass (SP), unplanted (U), and sterilized unplanted (SU) treatments showing groupings of microbial communities by treatment

5.0. Conclusions

Field specimens of many of the native California species growing in contaminated soil at the site were found to uptake PAHs, chlorinated dioxin/furans, silver, cadmium and lead in their roots and/or foliage (Table 5.1). Purple needlegrass, Blue Elderberry and Yerba Santa showed the most uptake of these contaminants. No uptake of PCBs or mercury was observed in the roots or foliage of any of the plant species tested. Compounds produced by some plants (phytogenic compounds) interfered with the analysis of petroleum hydrocarbons (EFH) and some PAHs, so it could not be determined if EFH compounds were taken up by plants in the field.

One of the primary goals of the field study was to identify plant species which would be most suitable for further research in the controlled greenhouse microcosm experiments. After completion of field data collection, Purple Needlegrass, Blue Elderberry, Mule Fat and Yerba Santa appeared to be the best species for phytoextraction of contaminants in the field (Table 5.1). However, the microcosm experiments were started before completion of all field testing due to budget and growing-season constraints. Based on preliminary screening data available at the time the greenhouse microcosms were planted, Coyote Brush, Mule Fat, and Purple Needlegrass were chosen for the microcosm experiments. Although Coyote Brush and Mule Fat were not the most ideal candidates for the microcosm study, they did show uptake of some contaminants, and the microcosm study did include Purple Needlegrass, which turned out to be one of the best plant species (of those tested) for uptake of PAHs and dioxin/furans (Table 5.1).

The controlled microcosm study showed that there was uptake of most of the contaminants in roots and foliage for one or more plant species. However, the plants did not significantly enhance the reduction of the COI concentrations in site soil, with the possible exception of chlorinated dioxin/furans.

Coyote Brush with and without fertilizer appeared to enhance the reduction of chlorinated dioxins/furan concentrations in microcosm soils. However, limited plant uptake of dioxins/furans was observed for Coyote Brush, and the published literature suggests that phytodegradation of dioxins/furans is unlikely (Campanella, Bock, and Schröder 2002). It is possible that the Coyote Brush is stimulating the microbial community in the rhizosphere to better degrade the dioxins/furans. Purple Needlegrass showed the greatest uptake of dioxins/furans into the foliage but did not reduce the dioxin/furan concentrations noticeably in the soil.

Table 5.1. The greatest contaminant concentrations observed in plant tissue from the field screening

Contaminant	Plant Species	Root Conc.	Foliage Conc.
PAHs	Blue Elderberry	1,740 µg/kg	not measured
	Yerba Santa	200 µg/kg	not measured
	Purple Needlegrass	703 µg/kg	not measured
Chlorinated Dioxins/Furans	Blue Elderberry	1,026 ng/kg	negligible
	Yerba Santa	0.42 µg/kg	0.90 µg/kg
	Purple Needlegrass	2.24 µg/kg	0.69 µg/kg
	Palmer's Goldenbush	0.43 µg/kg	0.76 µg/kg
Silver	Laurel Sumac	7.34 mg/kg	not detected
	Summer Mustard	1.43 mg/kg	0.405 mg/kg
Cadmium	Mule Fat	1.84 mg/kg	3.64 mg/kg
	Coyote Brush	1.52 mg/kg	2.12 mg/kg
Lead	Purple Needlegrass	8.92 mg/kg	1.17 mg/kg

Purple Needlegrass and Coyote Brush appeared to reduce soil concentrations of PCBs in the microcosms relative to the sterilized control. However, PCB may have adsorbed to the glass in the microcosm jars as indicated by similar reductions in PCB soil concentrations observed for the sterilized, unplanted controls. None of the plants significantly affected PAH concentrations relative to unplanted controls. For petroleum hydrocarbons, laboratory discrepancies in the EFH analyses precluded the ability to discern if plants enhanced biodegradation of petroleum hydrocarbons.

None of the plants were identified as hyper-accumulators of metals and none of the soil metal concentrations significantly decreased during the 211-day microcosms experiment. All of the metals were taken into the plants to some degree, with Purple Needlegrass showing the most promise for metal extraction as it showed some of the highest concentrations of all metals tested in roots and was the only species that contained mercury and silver in the foliage. The chelated Coyote Brush had significantly higher concentrations of lead in the roots and foliage than normal Coyote Brush, which may account for the poor plant health of the plants grown in chelated soil. Even though the metal concentrations in the soil were not reduced by the plants during this experiment, they could still potentially assist in remediation given longer time with more biomass.

There was a significant amount of variability in contaminant concentrations even after the homogenization of soil. This was particularly observed for PAHs, PCBs, and dioxins/furans, where one of the samples sometimes contained concentrations that were more than 50% greater than the other samples. The heterogeneity was also likely responsible for the apparent “increase” in concentrations observed for many of the COIs from Day 85 to Day 211 of the experiment.

The method of measuring volatilization did not detect any of the COIs coming off the plants growing in the greenhouse microcosms. Although mercury volatilization was not

tested, It is highly unlikely that any of the plants would volatilize mercury since little or no uptake of mercury into plant foliage was observed. Volatile compounds produced by the plants themselves (phytogenic compounds) were detected such as d-limonene, stearic and oleic acids, and stigmastan-3, 5-diene.

Given the limited decreases in soil COI concentrations observed in the greenhouse microcosm experiments, it is unlikely that phytoremediation will contribute significantly to remediation of soil contamination at the site over a short time. However, the time frame of the microcosm experiment was only 8 months, and given more time phytoremediation might possibly contribute to remediation of the COIs over the long-run. Phytoremediation could be employed for portions of the site with low COI concentrations where the length of time required for phytoremediation would not be an issue. Planting with native plants could likely be a part of site restoration efforts, and such plantings could provide long-term enhancements to other shorter-term COI remediation efforts.

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Appendix A: Graphs of Soil Microcosm COI Concentrations after 0, 85, and 211 Days

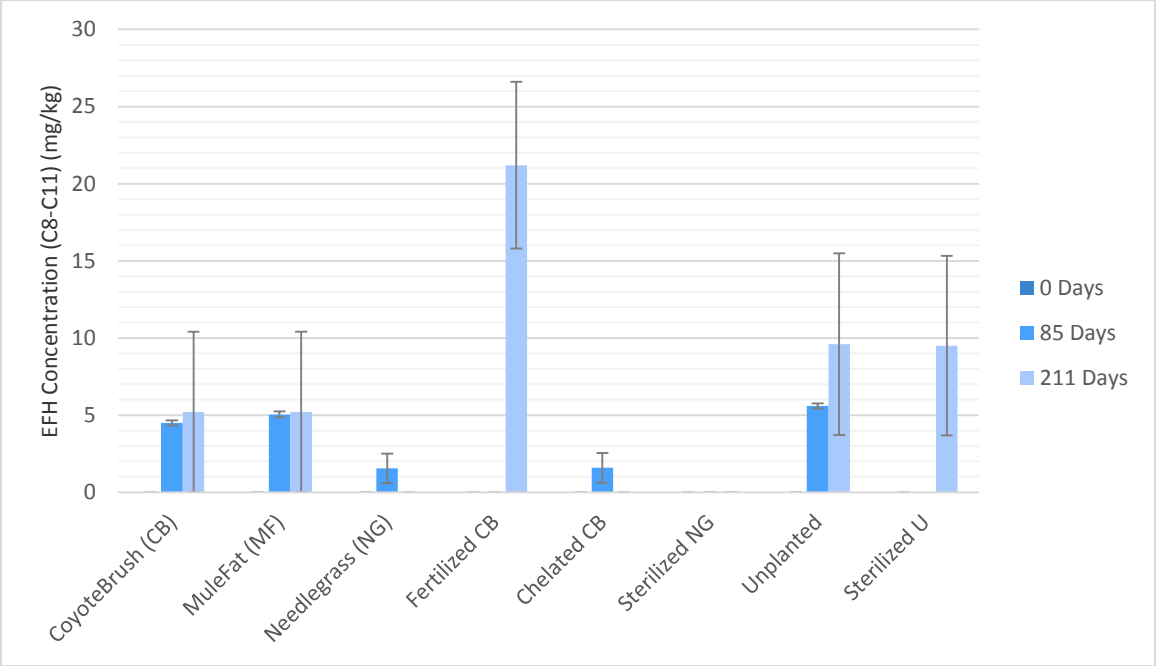

Figure A1: EFH (C8-C11) concentrations in microcosm soil after 0, 85, and 211 days

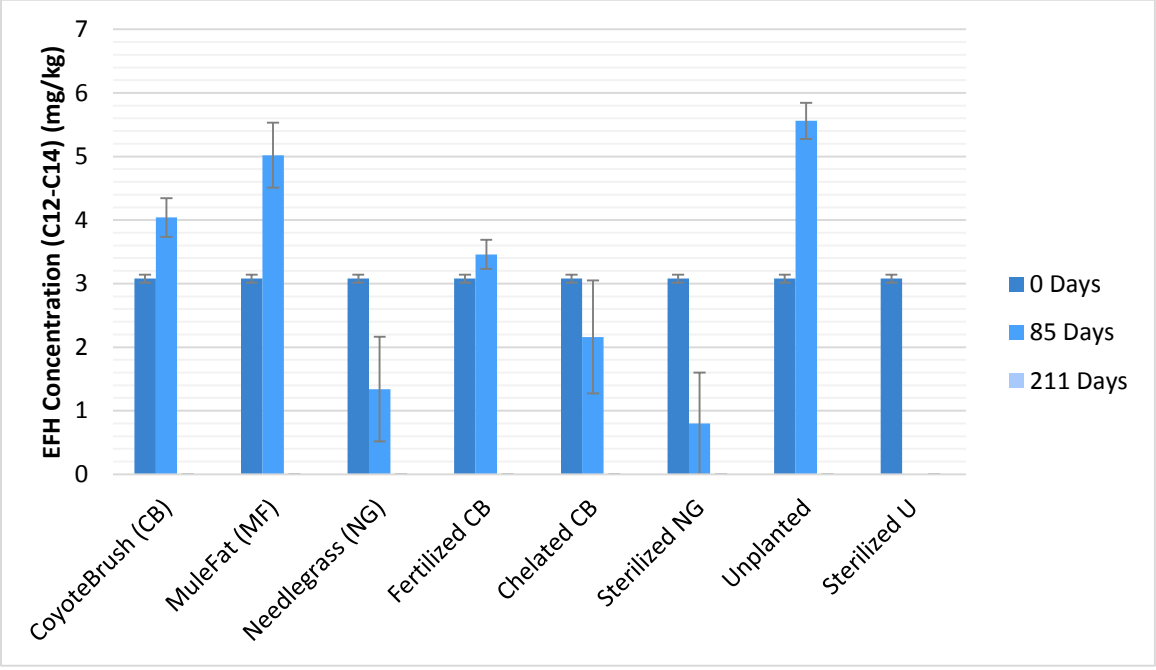

Figure A2: EFH (C12-C14) concentrations in microcosm soil after 0, 85, and 211 days

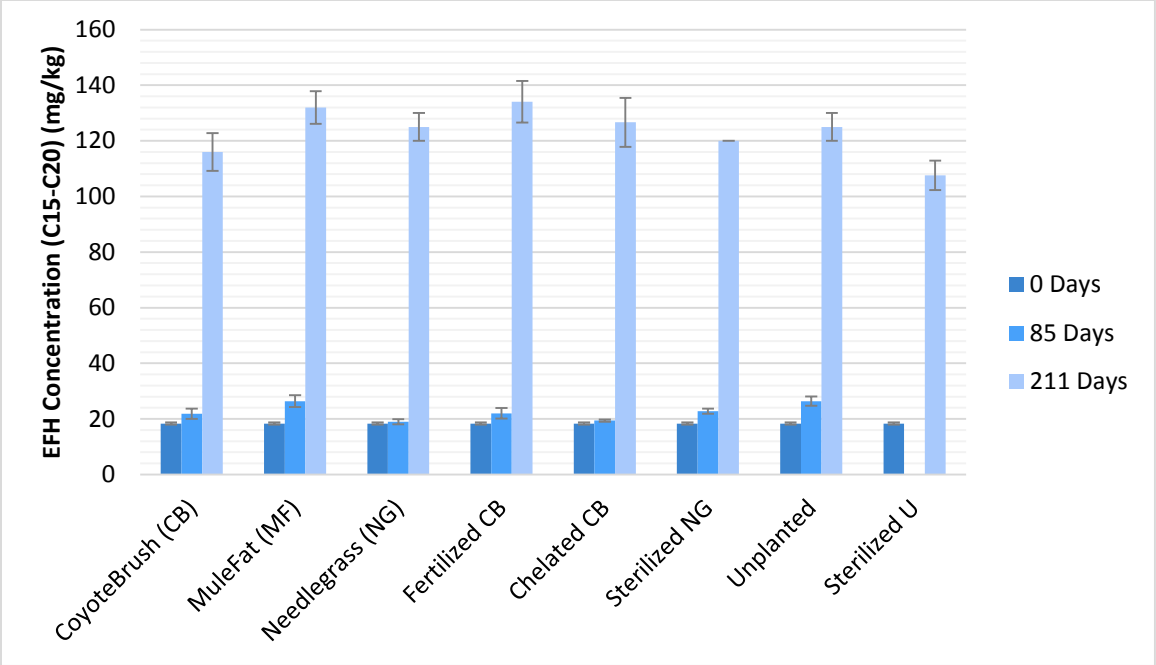

Figure A3: EFH (C15-C20) concentrations in microcosm soil after 0, 85, and 211 days

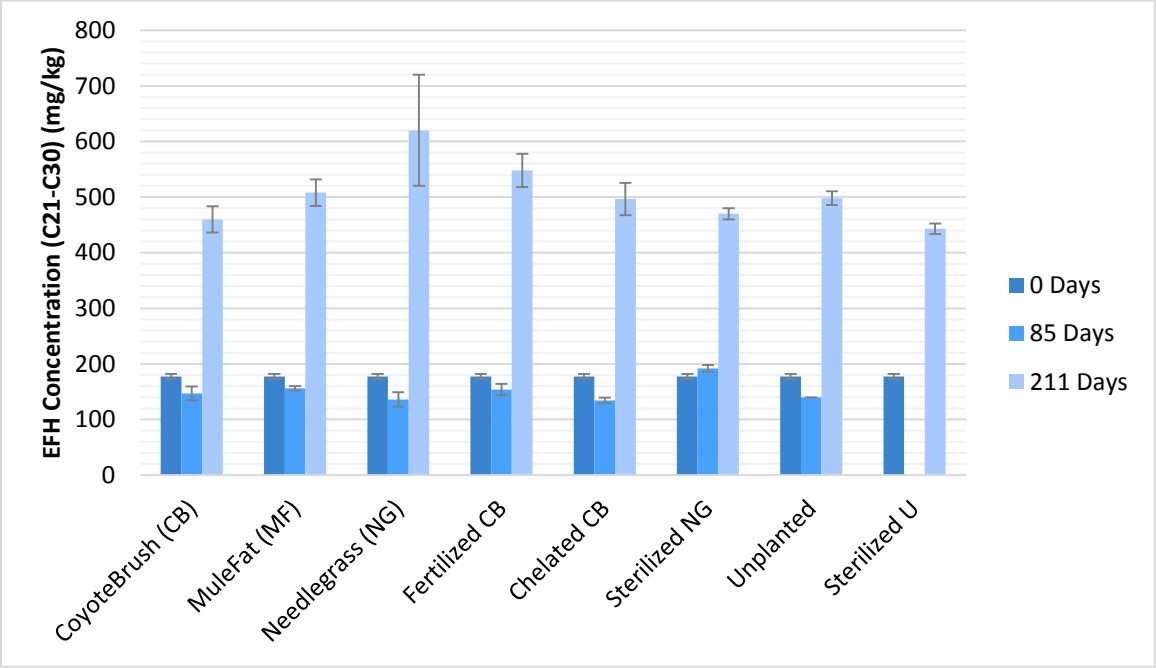

Figure A4: EFH (C21-C30) concentrations in microcosm soil after 0, 85, and 211 days

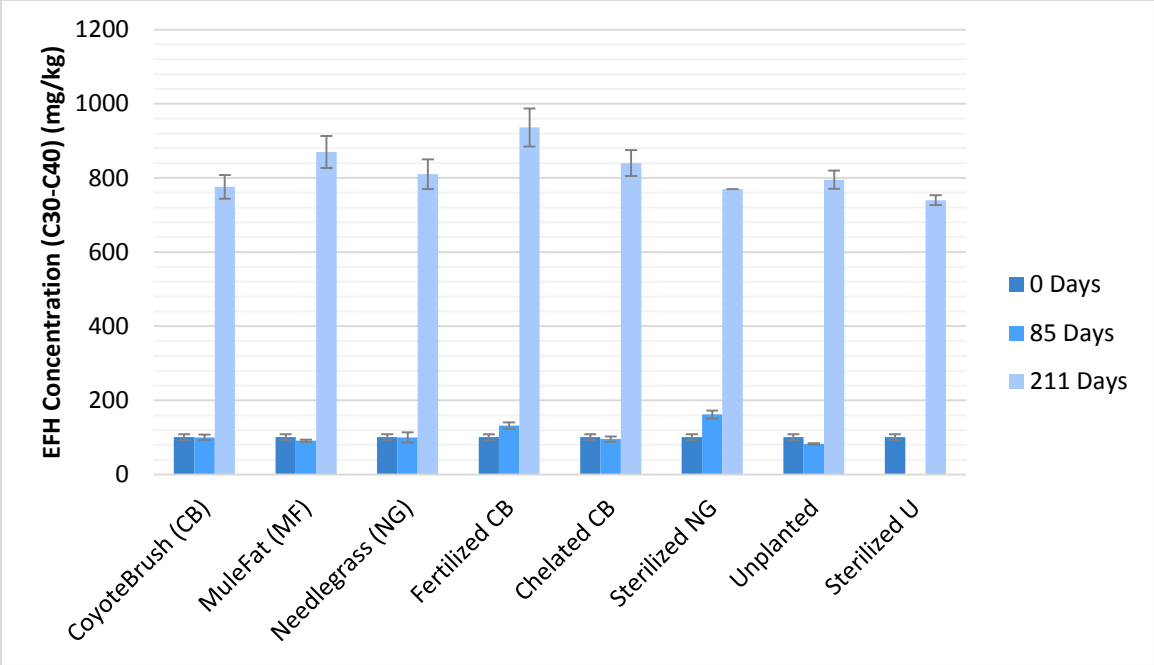

Figure A5: EFH (C30-C40) concentrations in microcosm soil after 0, 85, and 211 days

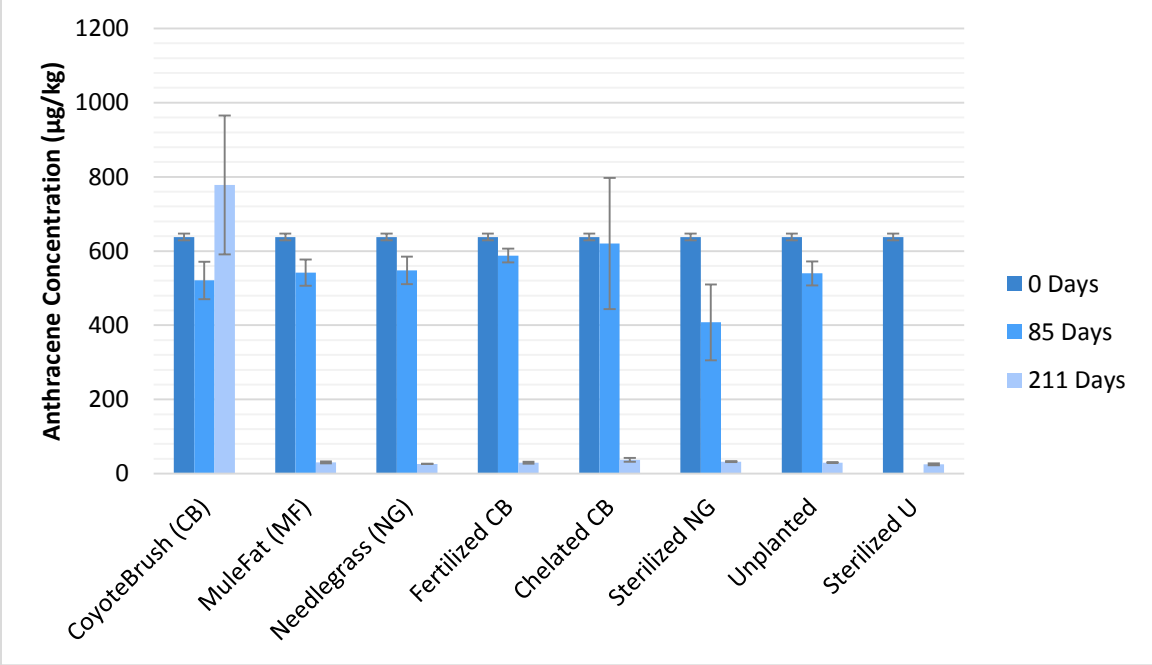

Figure A6: Anthracene concentrations in microcosm soil after 0, 85, and 211 days

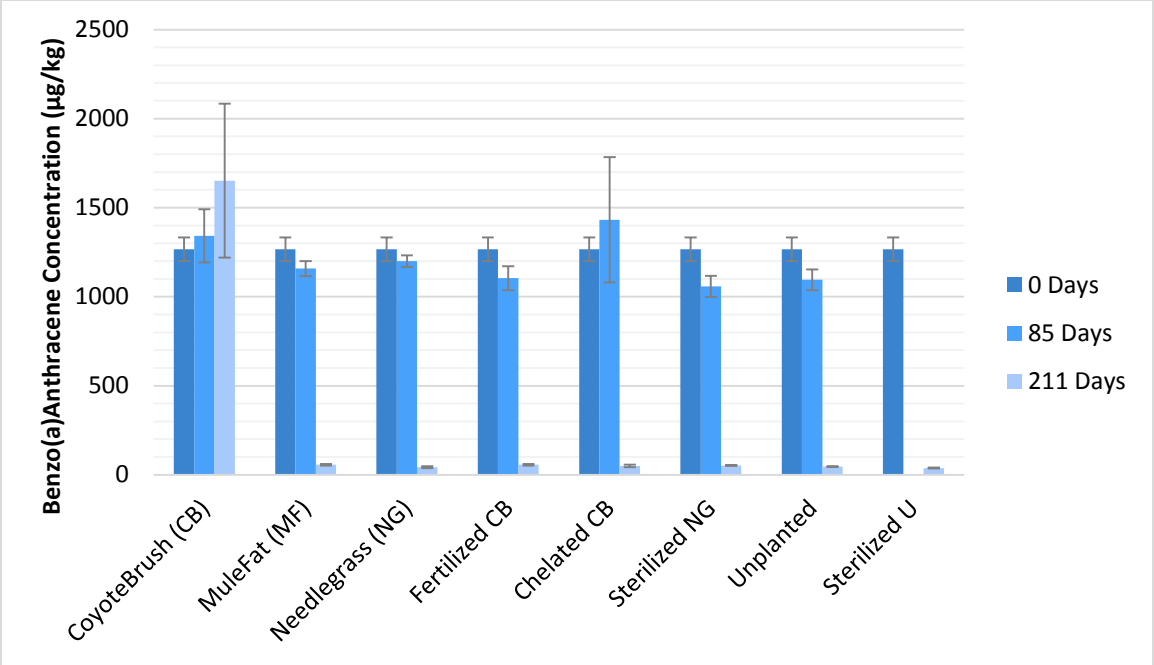

Figure A7: Benzo(a)anthracene concentrations in microcosm soil after 0, 85, and 211 days

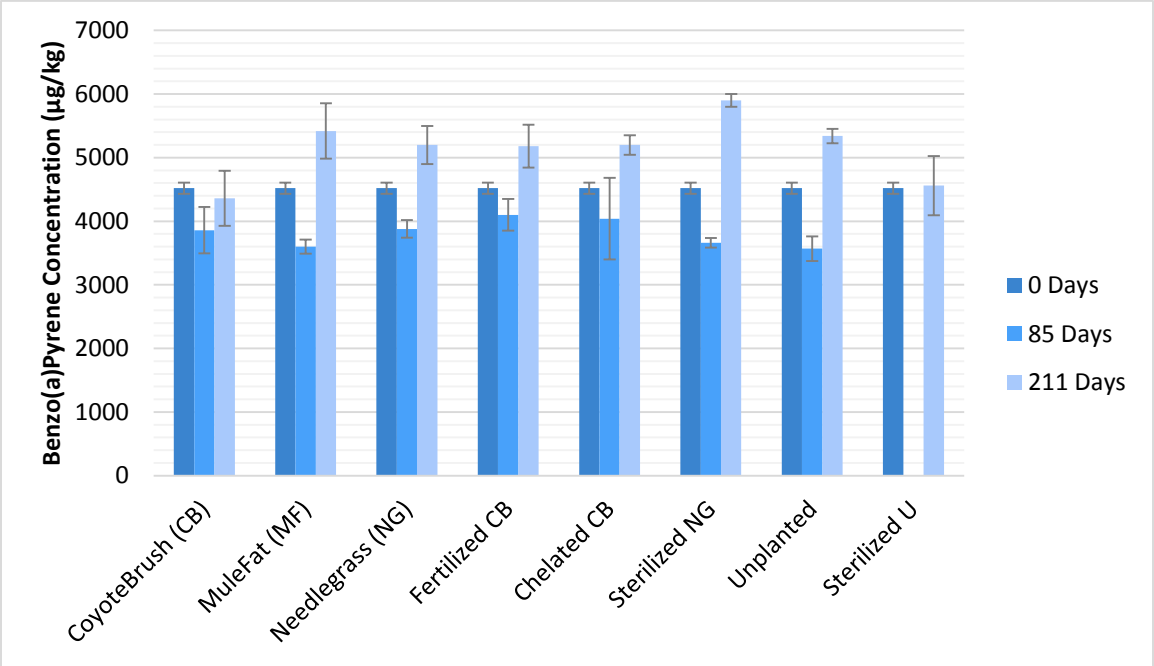

Figure A8: Benzo(a)pyrene concentrations in microcosm soil after 0, 85, and 211 days

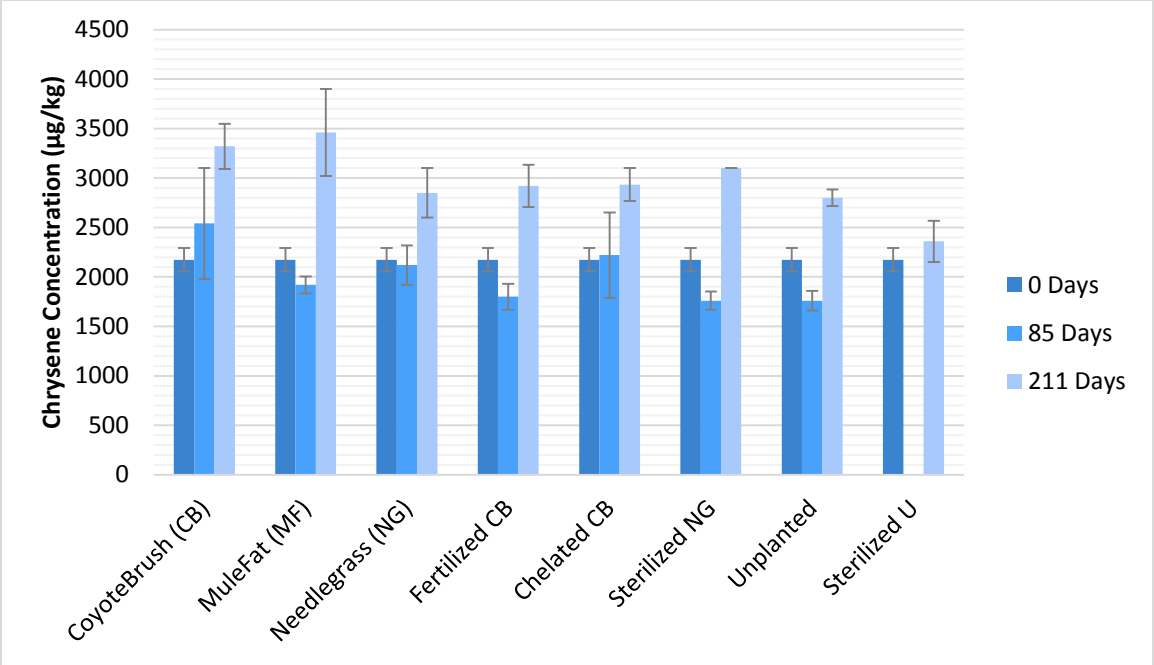

Figure A9: Chrysene concentrations in microcosm soil after 0, 85, and 211 days

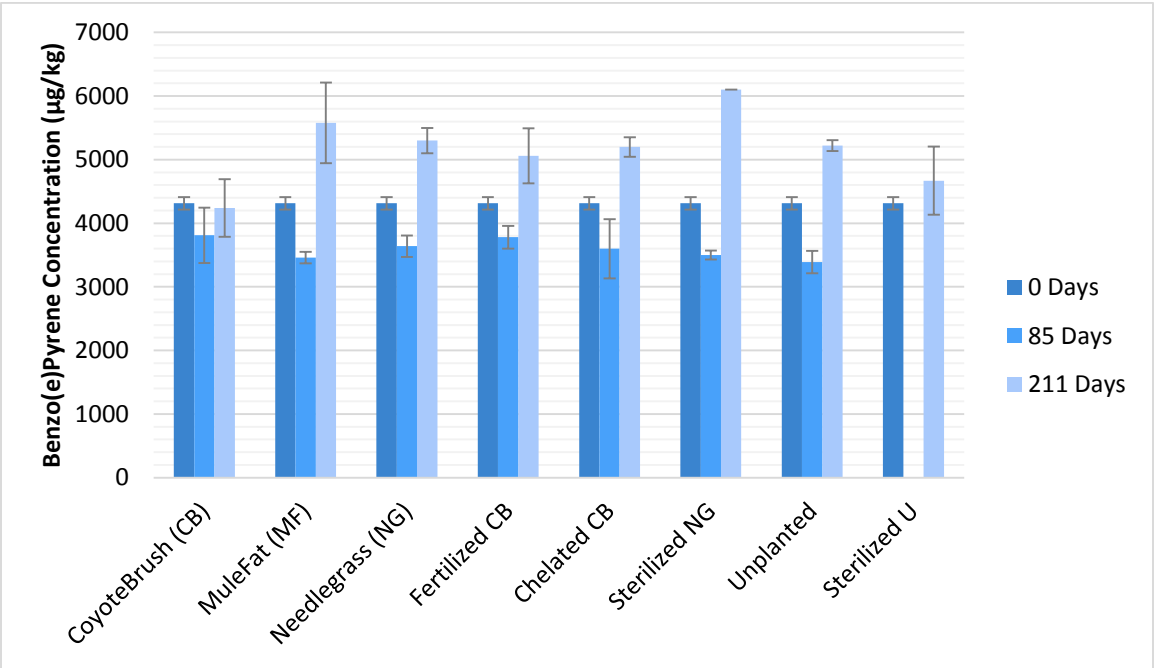

Figure A10: Benzo(e)pyrene concentrations in microcosm soil after 0, 85, and 211 days

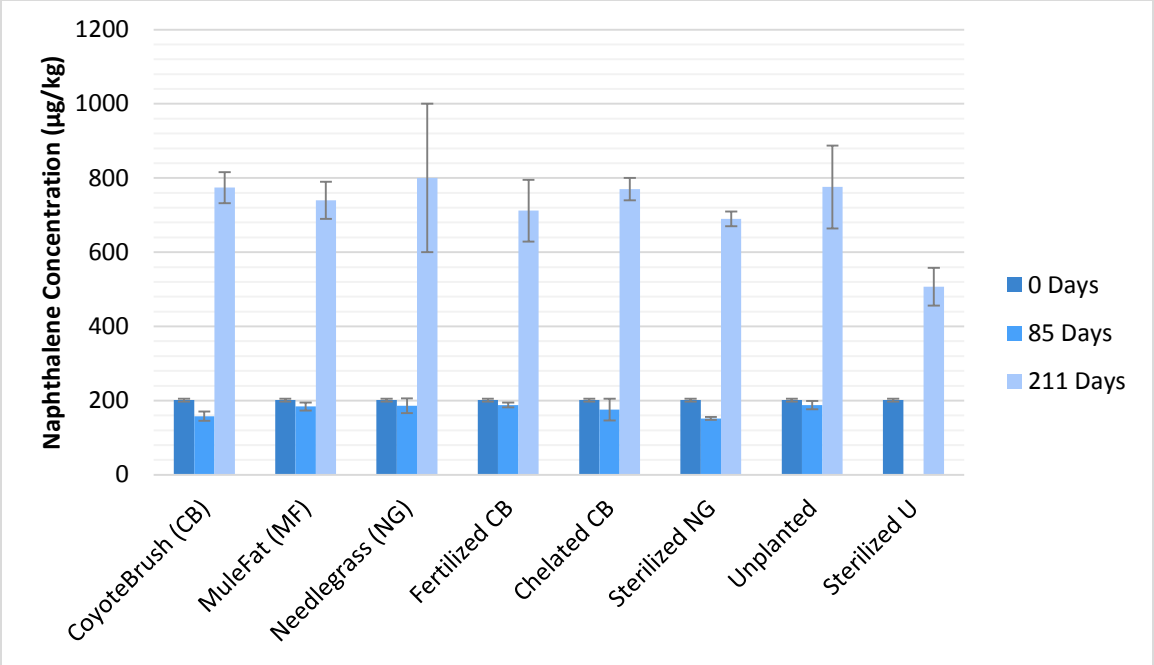

Figure A11: Naphthalene concentrations in microcosm soil after 0, 85, and 211 days

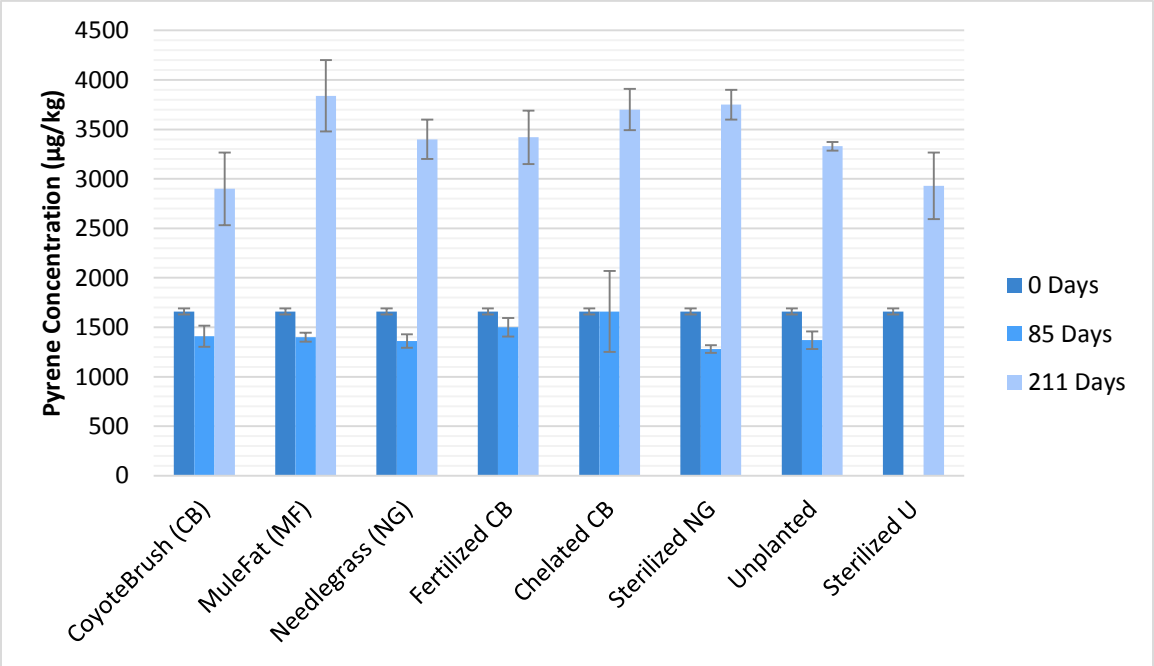

Figure A12: Pyrene concentrations in microcosm soil after 0, 85, and 211 days

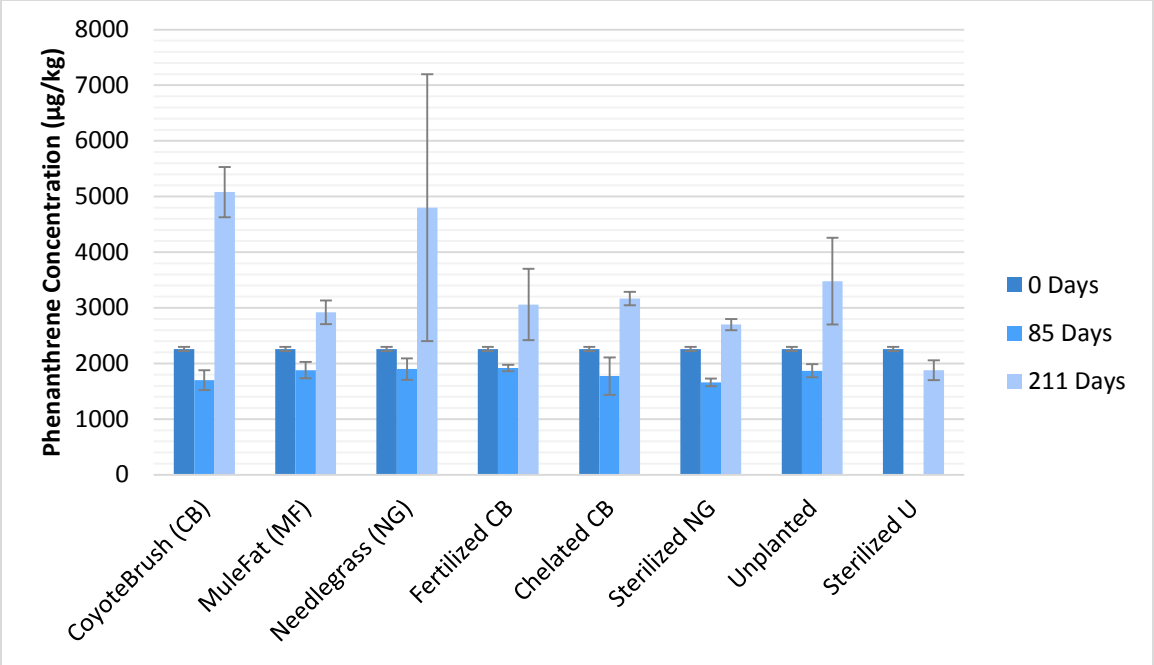

Figure A13: Phenanthrene concentrations in microcosm soil after 0, 85, and 211 days

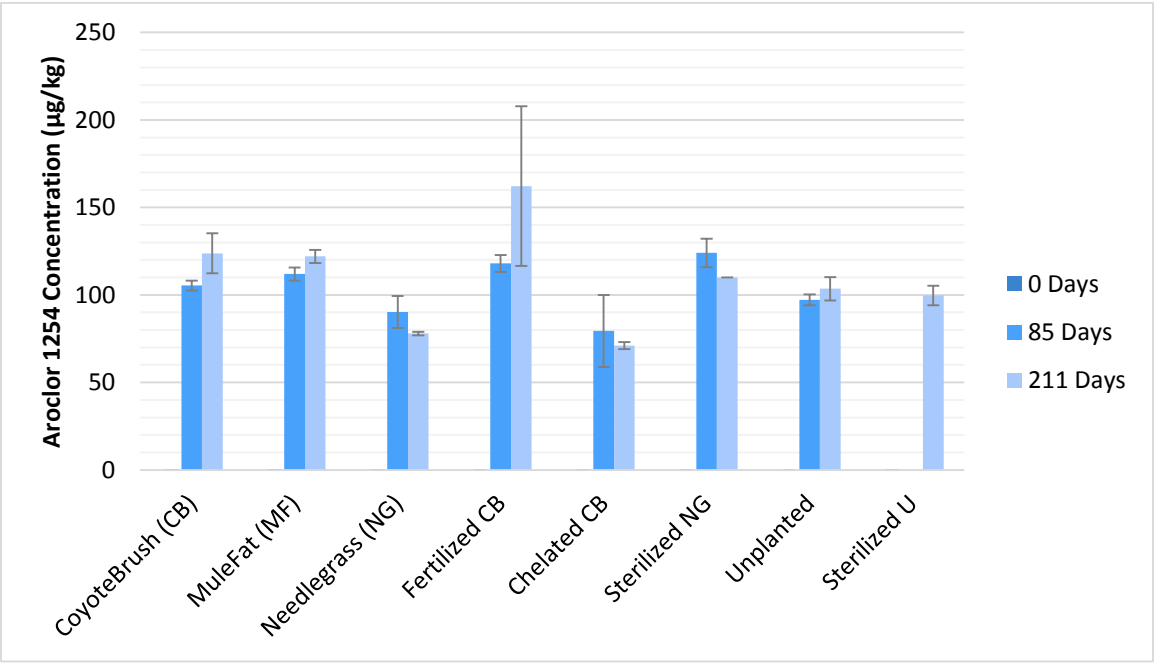

Figure A14: Aroclor 1254 concentrations in microcosm soil after 0, 85, and 211 days

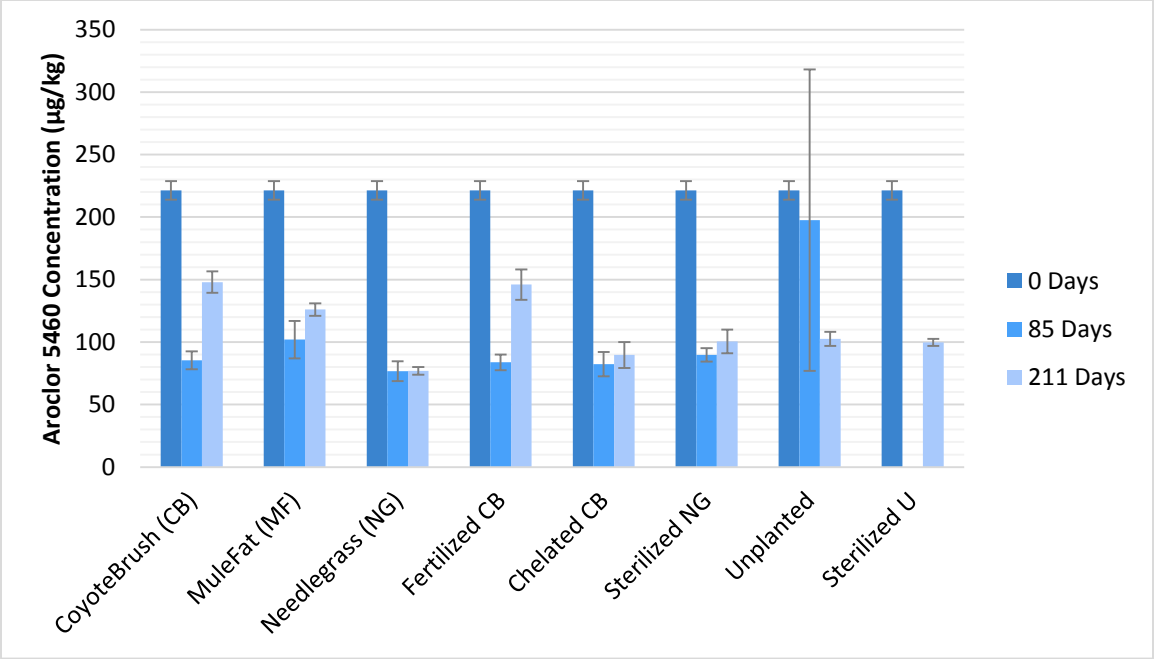

Figure A15: Aroclor 5460 concentrations in microcosm soil after 0, 85, and 211 days

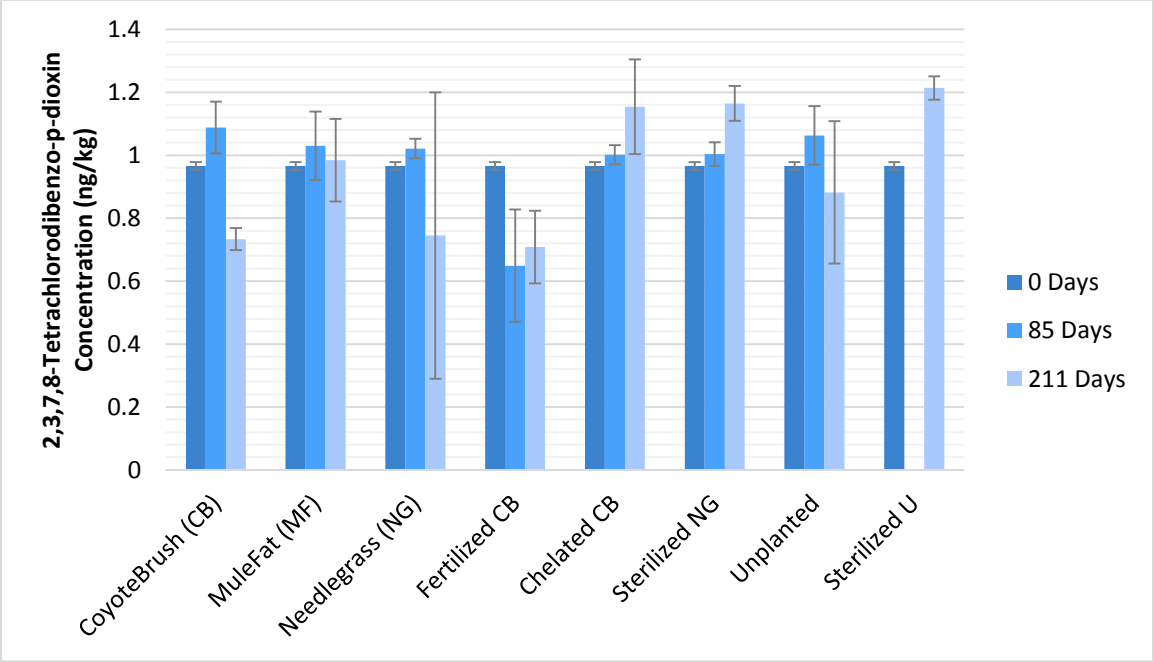

Figure A16: 2,3,7,8-Tetrachlorodibenzo-p-dioxin concentrations in microcosm soil after 0, 85, and 211 days

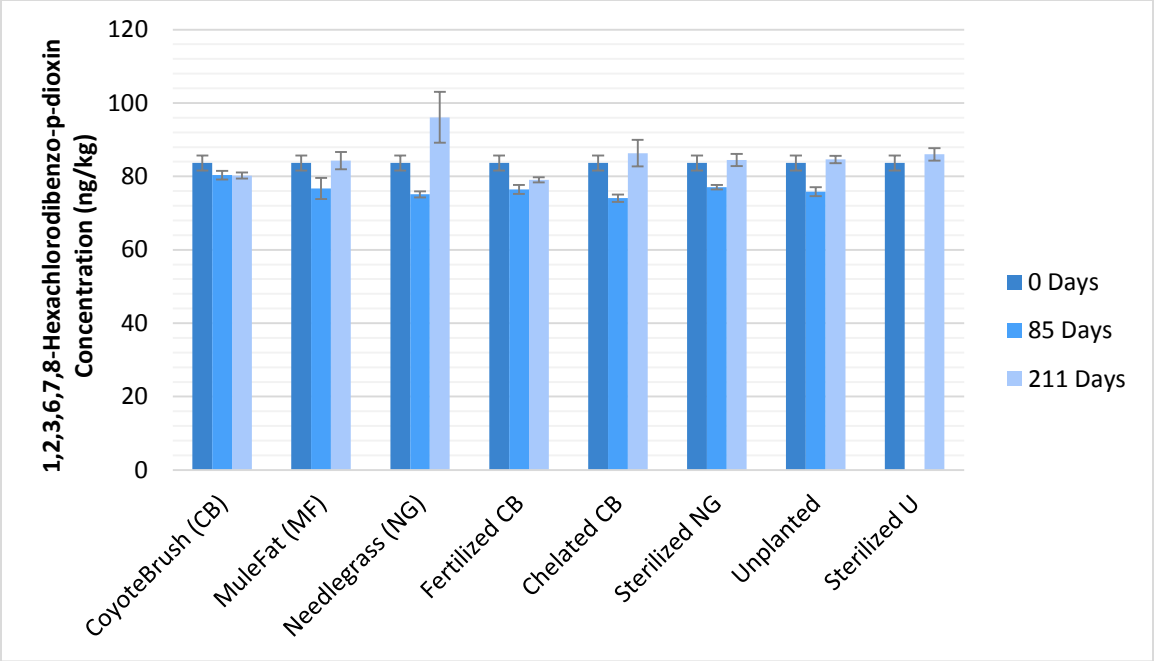

Figure A17: 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin concentrations in microcosm soil after 0, 85, and 211 days

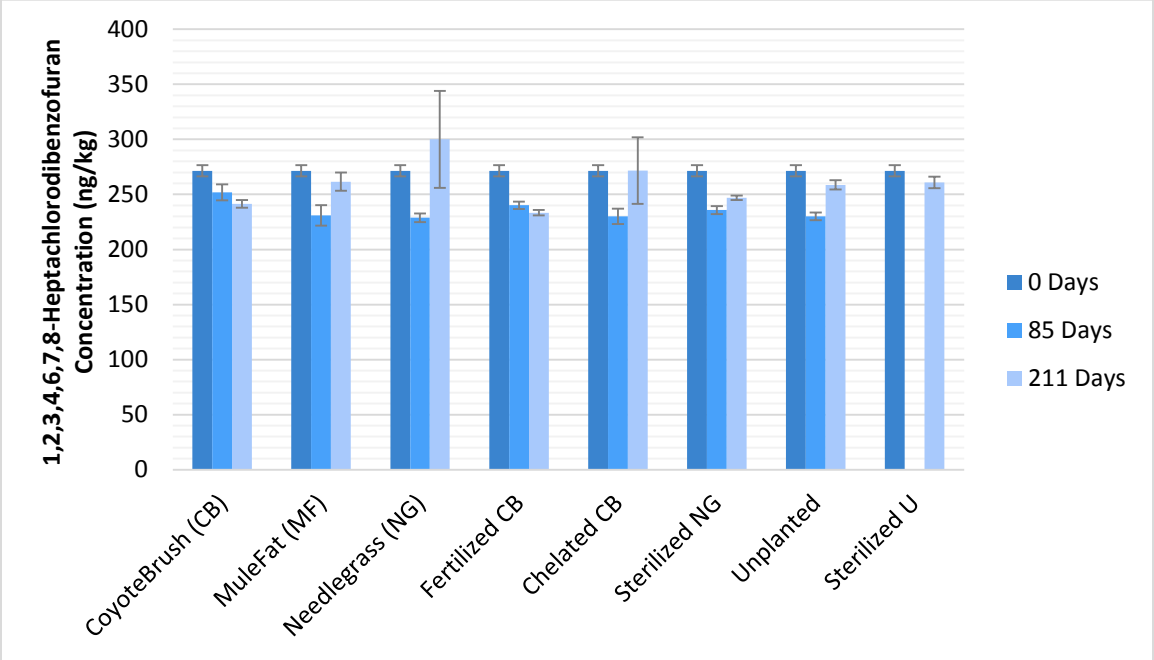

Figure A18: 1,2,3,4,6,7,8-Heptachlorodibenzofuran concentrations in microcosm soil after 0, 85, and 211 days

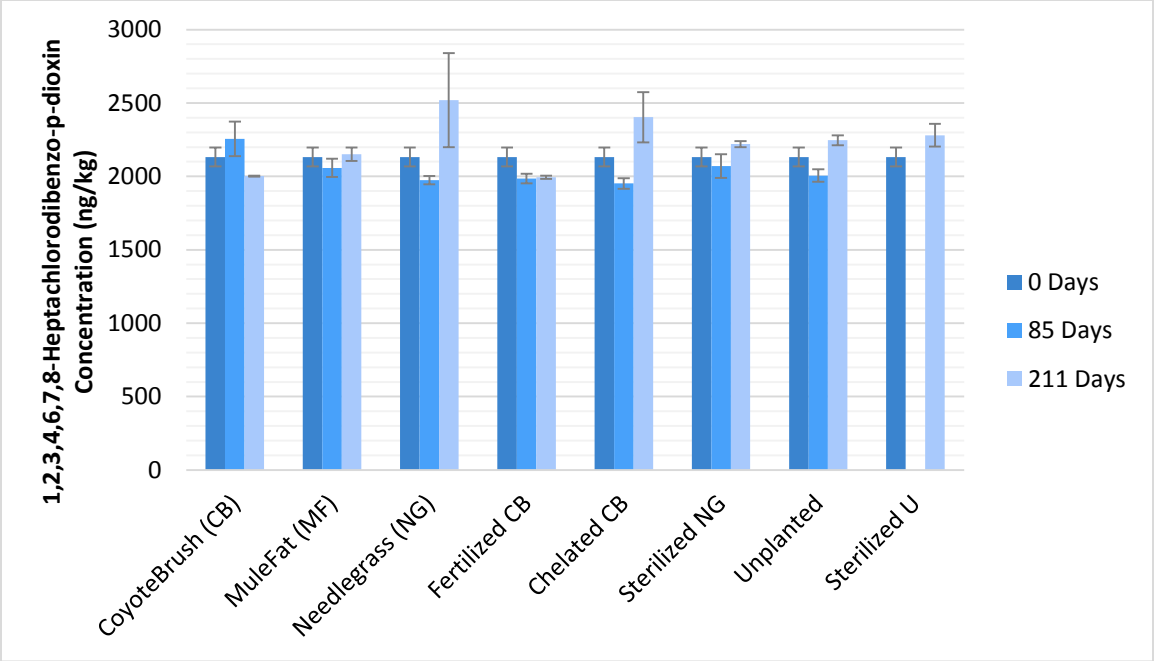

Figure A19: 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin concentrations in microcosm soil after 0, 85, and 211 days

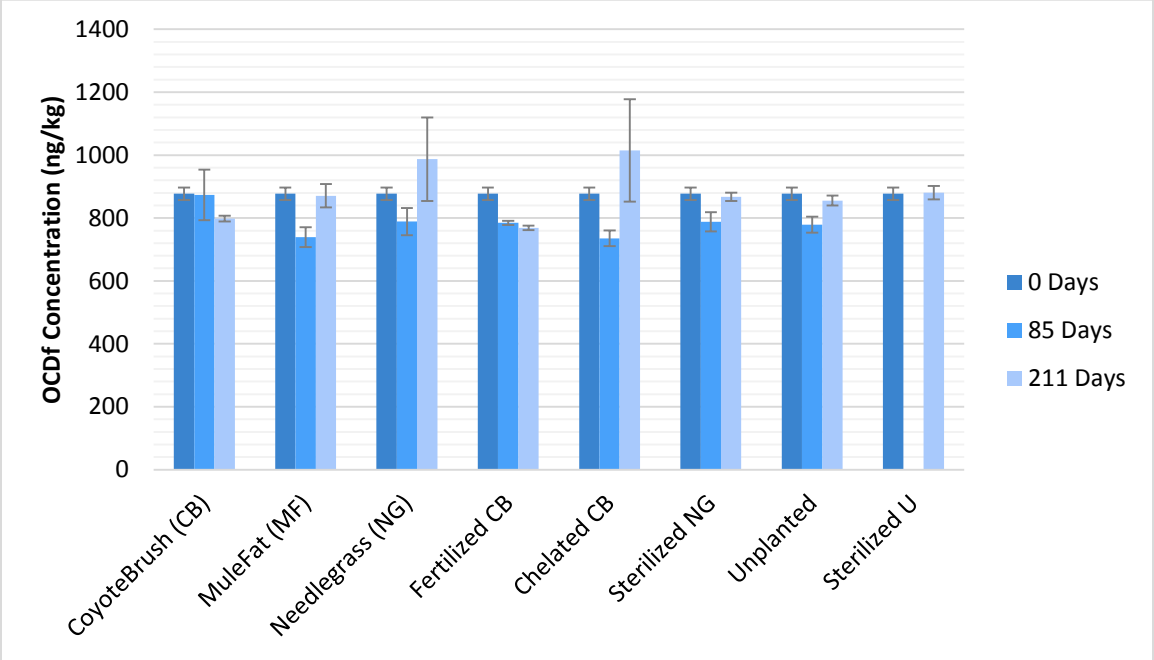

Figure A20: OCDF concentrations in microcosm soil after 0, 85, and 211 days

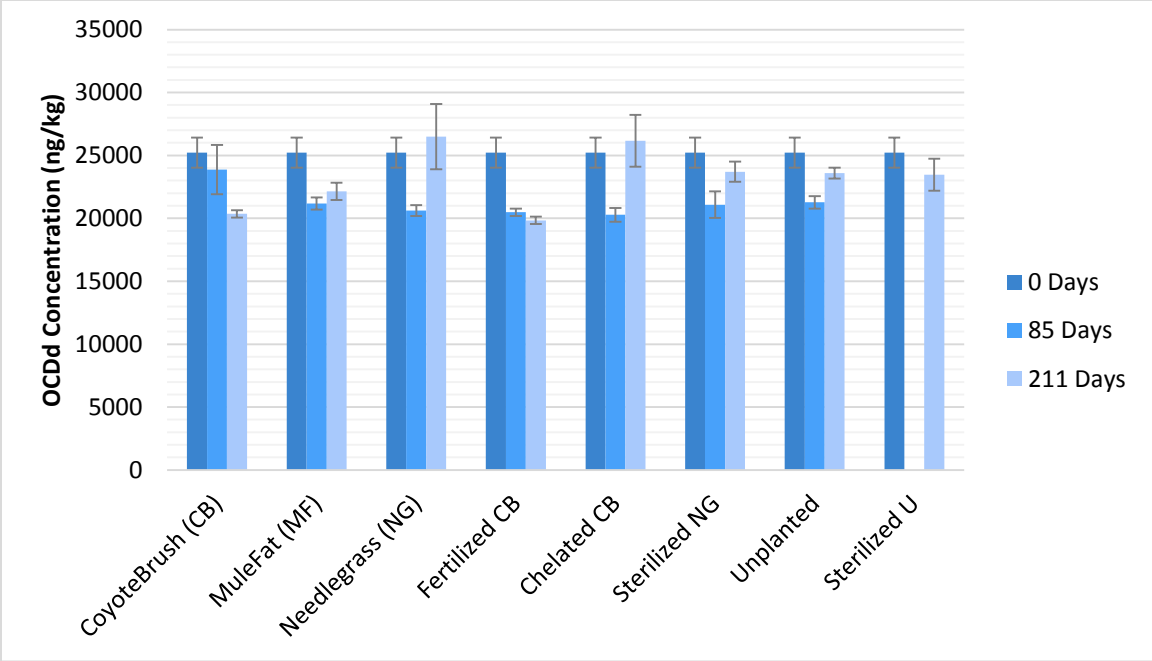

Figure A21: OCDD concentrations in microcosm soil after 0, 85, and 211 days

Appendix B: Statistical Analysis on Microcosm Experiment Results

One-way ANOVA: Total EFH versus Treatment

```
Source DF SS MS F P
Treatment 7 396931 56704 2.81 0.027
Error 24 484128 20172
Total 31 881060

S = 142.0 R-Sq = 45.05% R-Sq(adj) = 29.02%


 Individual 95% CIs For Mean Based on
 Pooled StDev
Level N Mean StDev --------+---------+---------+---------+-
CPA 3 1463.3 123.4 (---------*----------)
FPA 5 1639.2 206.7 (-------*--------)
PA 5 1357.2 145.3 (-------*-------)
PB 5 1515.2 165.6 (-------*-------)
PC 2 1555.0 205.1 (------------*------------)
SP 2 1360.0 14.1 (------------*------------)
SU 5 1300.1 57.8 (-------*-------)
U 5 1427.6 90.9 (-------*-------)
 --------+---------+---------+---------+-
 1280 1440 1600 1760

Pooled StDev = 142.0


Grouping Information Using Tukey Method

Treatment N Mean Grouping
FPA 5 1639.2 A
PC 2 1555.0 A B
PB 5 1515.2 A B
CPA 3 1463.3 A B
U 5 1427.6 A B
SP 2 1360.0 A B
PA 5 1357.2 A B
SU 5 1300.1 B
```

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.71%

One-way ANOVA: Sum of PAHs versus Treatment

```
Source DF SS MS F P
Treatment 7 784715889 112102270 2.02 0.094
Error 24 1331621322 55484222
Total 31 2116337212

S = 7449 R-Sq = 37.08% R-Sq(adj) = 18.73%


 Individual 95% CIs For Mean Based on
 Pooled StDev
Level N Mean StDev ----+---------+---------+---------+-----
CPA 3 46567 3904 (----------*----------)
FPA 5 45014 8649 (-------*--------)
PA 5 51985 6018 (--------*--------)
PB 5 49412 10580 (--------*-------)
PC 2 46234 9448 (-------------*------------)
SP 2 46350 1394 (-------------*-------------)
SU 5 35630 7596 (--------*-------)
U 5 44622 4671 (--------*-------)
 ----+---------+---------+---------+-----
 32000 40000 48000 56000

Pooled StDev = 7449
```

Results for: Soil Day 85 no SU.MTW

One-way ANOVA: Sum of PAHs versus Treatment

```
Source DF SS MS F P
Treatment 6 117344978 19557496 0.41 0.868
Error 28 1347692627 48131880
Total 34 1465037605

S = 6938 R-Sq = 8.01% R-Sq(adj) = 0.00%


 Individual 95% CIs For Mean Based on
```

```
 Pooled StDev
Level N Mean StDev ---------+---------+---------+---------+
CPA 5 38232 14360 (-----------*-----------)
FPA 5 42952 4013 (-----------*-----------)
PA 5 36956 8057 (-----------*-----------)
PB 5 37609 3564 (-----------*-----------)
PC 5 38132 1945 (-----------*-----------)
SP 5 38185 1691 (-----------*-----------)
U 5 38037 5509 (-----------*-----------)
 ---------+---------+---------+---------+
 35000 40000 45000 50000

Pooled StDev = 6938


Grouping Information Using Tukey Method

Treatment N Mean Grouping
FPA 5 42952 A
CPA 5 38232 A
SP 5 38185 A
PC 5 38132 A
U 5 38037 A
PB 5 37609 A
PA 5 36956 A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.64%
```

Two-Sample T-Test and CI: Total PAH Conc. (roots), Treatment

```
Two-sample T for Total PAH Conc. (roots)

Treatment N Mean StDev SE Mean
PC 2 10338 1616 1143
```

SP 2 12333 1750 1237

Difference = mu (PC) - mu (SP)
Estimate for difference: -1995
95% CI for difference: (-9243, 5254)
T-Test of difference = 0 (vs not =): T-Value = -1.18 P-Value = 0.358 DF = 2
Both use Pooled StDev = 1684.5935

One-way ANOVA: Aroclor 1260 versus Treatment

Source DF SS MS F P
Treatment 7 12503 1786 17.31 0.000
Error 24 2477 103
Total 31 14979

S = 10.16 R-Sq = 83.47% R-Sq(adj) = 78.64%

Individual 95% CIs For Mean Based on
Pooled StDev
Level N Mean StDev --------+---------+---------+---------+-
CPA 3 67.67 3.06 (----*----)
FPA 5 130.00 12.25 (---*---)
PA 5 118.00 10.95 (---*---)
PB 5 113.80 11.71 (---*--)
PC 2 70.50 2.12 (-----*-----)
SP 2 96.00 5.66 (-----*-----)
SU 5 88.30 6.38 (--*---)
U 5 98.00 12.55 (---*---)
--------+---------+---------+---------+-
75 100 125 150

Pooled StDev = 10.16

Grouping Information Using Tukey Method

Treatment N Mean Grouping
FPA 5 130.00 A

PA 5 118.00 A B
PB 5 113.80 A B
U 5 98.00 B C
SP 2 96.00 B C D
SU 5 88.30 C D
PC 2 70.50 C D
CPA 3 67.67 D

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.71%

Results for: Initial vs Final U.MTW

Two-Sample T-Test and CI: Aroclor 1260, Time (days)

Two-sample T for Aroclor 1260

Time (days)	N	Mean	StDev	SE Mean
0	15	139.33	9.61	2.5
211	5	98.0	12.5	5.6

Difference = mu (0) - mu (211)
Estimate for difference: 41.33
95% CI for difference: (30.12, 52.55)
T-Test of difference = 0 (vs not =): T-Value = 7.74 P-Value = 0.000 DF = 18
Both use Pooled StDev = 10.3369

Results for: Initial vs Final SU.MTW

Two-Sample T-Test and CI: Aroclor 1260, Time (days)

Two-sample T for Aroclor 1260

```
Time
(days) N Mean StDev SE Mean
 0 15 139.33 9.61 2.5
211 5 88.30 6.38 2.9


Difference = mu ( 0) - mu (211)
Estimate for difference: 51.03
95% CI for difference: (41.28, 60.79)
T-Test of difference = 0 (vs not =): T-Value = 10.99 P-Value = 0.000 DF = 18
Both use Pooled StDev = 8.9942
```

Results for: Initial vs Final SP.MTW

Two-Sample T-Test and CI: Aroclor 1260, Time (days)

```
Two-sample T for Aroclor 1260

Time
(days) N Mean StDev SE Mean
 0 15 139.33 9.61 2.5
211 2 96.00 5.66 4.0


Difference = mu ( 0) - mu (211)
Estimate for difference: 43.33
95% CI for difference: (28.25, 58.42)
T-Test of difference = 0 (vs not =): T-Value = 6.12 P-Value = 0.000 DF = 15
Both use Pooled StDev = 9.3998
```

Results for: Initial vs Final PC.MTW

Two-Sample T-Test and CI: Aroclor 1260, Time (days)

```
Two-sample T for Aroclor 1260

Time
(days) N Mean StDev SE Mean
 0 15 139.33 9.61 2.5
211 2 70.50 2.12 1.5
```

```
Difference = mu ( 0) - mu (211)
Estimate for difference: 68.83
95% CI for difference: (53.91, 83.76)
T-Test of difference = 0 (vs not =): T-Value = 9.83 P-Value = 0.000 DF = 15
Both use Pooled StDev = 9.3017
```

Results for: Initial vs Final CPA.MTW

Two-Sample T-Test and CI: Aroclor 1260, Time (days)

```
Two-sample T for Aroclor 1260

Time
(days) N Mean StDev SE Mean
 0 15 139.33 9.61 2.5
211 3 67.67 3.06 1.8


Difference = mu ( 0) - mu (211)
Estimate for difference: 71.67
95% CI for difference: (59.53, 83.81)
T-Test of difference = 0 (vs not =): T-Value = 12.51 P-Value = 0.000 DF = 16
Both use Pooled StDev = 9.0554
```

One-way ANOVA: Total Dioxins/Furans versus Treatment

```
Source DF SS MS F P
Treatment 7 179490657 25641522 5.51 0.001
Error 24 111653300 4652221
Total 31 291143957

S = 2157 R-Sq = 61.65% R-Sq(adj) = 50.46%

 Individual 95% CIs For Mean Based on Pooled StDev
Level N Mean StDev +---------+---------+---------+---------
CPA 3 30075 4103 (------*------)
FPA 5 23038 702 (-----*-----)
PA 5 23605 653 (----*-----)
PB 5 25643 1631 (----*-----)
```

```
PC 2 30558 4421 (--------*--------)
SP 2 27253 1178 (--------*--------)
SU 5 27118 3095 (----*-----)
U 5 27181 1053 (-----*----)
 +---------+---------+---------+---------
 21000 24500 28000 31500

Pooled StDev = 2157

Grouping Information Using Tukey Method

Treatment N Mean Grouping
PC 2 30558 A
CPA 3 30075 A
SP 2 27253 A B
U 5 27181 A B
SU 5 27118 A B
PB 5 25643 A B
PA 5 23605 B
FPA 5 23038 B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.71%
```

Two-Sample T-Test and CI: Total Dioxins/Furans, Treatment

```
Two-sample T for Total Dioxins/Furans

Treatment N Mean StDev SE Mean
PA 5 23605 653 292
U 5 27181 1053 471


Difference = mu (PA) - mu (U)
```

Estimate for difference: -3576

95% CI for difference: (-4855, -2298)

T-Test of difference = 0 (vs not =): T-Value = -6.45 P-Value = 0.000 DF = 8

Both use Pooled StDev = 876.3355

Two-Sample T-Test and CI: Total Dioxins/Furans, Treatment

Two-sample T for Total Dioxins/Furans

Treatment	N	Mean	StDev	SE Mean
PB	5	25643	1631	729
U	5	27181	1053	471

Difference = mu (PB) - mu (U)

Estimate for difference: -1538

95% CI for difference: (-3540, 464)

T-Test of difference = 0 (vs not =): T-Value = -1.77 P-Value = 0.114 DF = 8

Both use Pooled StDev = 1372.6487

Two-Sample T-Test and CI: Total Dioxin/Furan Conc. (roots, Treatment

Two-sample T for Total Dioxin/Furan Conc. (roots

Treatment	N	Mean	StDev	SE Mean
PC	2	9566	8292	5863
SP	2	6714	1607	1137

Difference = mu (PC) - mu (SP)

Estimate for difference: 2852

95% CI for difference: (-22845, 28549)

T-Test of difference = 0 (vs not =): T-Value = 0.48 P-Value = 0.680 DF = 2

Both use Pooled StDev = 5972.4237

Results for: Initial vs Final PA.MTW

One-way ANOVA: Total Dioxins/Furans versus Time (days)

```
Source DF SS MS F P
Time (days) 1 97979954 97979954 5.12 0.036
Error 18 344334352 19129686
Total 19 442314306

S = 4374 R-Sq = 22.15% R-Sq(adj) = 17.83%


 Individual 95% CIs For Mean Based on
 Pooled StDev
Level N Mean StDev -----+---------+---------+---------+----
 0 15 28716 4947 (-------*-------)
211 5 23605 653 (-------------*------------)
 -----+---------+---------+---------+----
 21000 24000 27000 30000

Pooled StDev = 4374
```

*Normality not met because of high dioxin concentration for one bulk soil replicate. However subsequent test omitting this replicate meets normality and is significant.

Results for: Initial vs Final FPA.MTW

Two-Sample T-Test and CI: Total Dioxins/Furans, Time (days)

```
Two-sample T for Total Dioxins/Furans

Time
(days) N Mean StDev SE Mean
 0 15 28716 4947 1277
211 5 23038 702 314


Difference = mu ( 0) - mu (211)
Estimate for difference: 5678
95% CI for difference: (931, 10425)
T-Test of difference = 0 (vs not =): T-Value = 2.51 P-Value = 0.022 DF = 18
Both use Pooled StDev = 4375.4326
```

Two-Sample T-Test and CI: Mercury, Time (days) (Sterilized Unplanted)

```
Two-sample T for Mercury

Time
(days) N Mean StDev SE Mean
 0 15 0.571 0.111 0.029
211 5 0.4936 0.0215 0.0096

Difference = mu ( 0) - mu (211)
Estimate for difference: 0.0777
95% CI for difference: (-0.0289, 0.1844)
T-Test of difference = 0 (vs not =): T-Value = 1.53 P-Value = 0.143 DF = 18
Both use Pooled StDev = 0.0983
```

*Note: normality not met because of a large mercury value of 0.953 in one sample of bulk soil

One-way ANOVA: Mercury versus Treatment

```
Source DF SS MS F P
Treatment 7 0.012039 0.001720 1.92 0.110
Error 24 0.021477 0.000895
Total 31 0.033517

S = 0.02991 R-Sq = 35.92% R-Sq(adj) = 17.23%


 Individual 95% CIs For Mean Based on
 Pooled StDev
Level N Mean StDev -----+---------+---------+---------+----
CPA 3 0.53133 0.00907 (-----------*-----------)
FPA 5 0.53480 0.04289 (--------*--------)
PA 5 0.55420 0.04038 (--------*--------)
PB 5 0.51120 0.02066 (--------*---------)
PC 2 0.53450 0.01202 (-------------*--------------)
SP 2 0.50850 0.01909 (-------------*--------------)
SU 5 0.49360 0.02155 (---------*--------)
U 5 0.53590 0.02899 (---------*--------)
 -----+---------+---------+---------+----
 0.480 0.510 0.540 0.570
```

Pooled StDev = 0.02991

Grouping Information Using Tukey Method

Treatment N Mean Grouping
PA 5 0.55420 A
U 5 0.53590 A
FPA 5 0.53480 A
PC 2 0.53450 A
CPA 3 0.53133 A
PB 5 0.51120 A
SP 2 0.50850 A
SU 5 0.49360 A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.71%

One-way ANOVA: Silver Conc. (roots) versus Treatment

Source DF SS MS F P
Treatment 5 19.664 3.933 12.78 0.000
Error 16 4.924 0.308
Total 21 24.588

S = 0.5548 R-Sq = 79.97% R-Sq(adj) = 73.72%

Individual 95% CIs For Mean Based on
Pooled StDev
Level N Mean StDev -------+---------+---------+---------+--
CPA 3 1.6900 0.4976 (---*----)
FPA 5 1.4174 0.5053 (--*---)
PA 5 1.3978 0.6964 (--*---)

```
PB 5 1.0410 0.4286 (---*--)
PC 2 4.5850 0.0354 (-----*----)
SP 2 1.7050 0.8556 (----*-----)
 -------+---------+---------+---------+--
 1.5 3.0 4.5 6.0

Pooled StDev = 0.5548


Grouping Information Using Tukey Method

Treatment N Mean Grouping
PC 2 4.5850 A
SP 2 1.7050 B
CPA 3 1.6900 B
FPA 5 1.4174 B
PA 5 1.3978 B
PB 5 1.0410 B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.47%
```

Two-Sample T-Test and CI: Silver Conc. (roots), Treatment

```
Two-sample T for Silver Conc. (roots)

Treatment N Mean StDev SE Mean
CPA 3 1.690 0.498 0.29
PA 5 1.398 0.696 0.31


Difference = mu (CPA) - mu (PA)
Estimate for difference: 0.292
95% CI for difference: (-0.846, 1.431)
```

T-Test of difference = 0 (vs not =): T-Value = 0.63 P-Value = 0.553 DF = 6
Both use Pooled StDev = 0.6370

One-way ANOVA: Cadmium versus Treatment

Source DF SS MS F P
Treatment 7 0.0853 0.0122 0.49 0.834
Error 24 0.5989 0.0250
Total 31 0.6841

S = 0.1580 R-Sq = 12.47% R-Sq(adj) = 0.00%

Individual 95% CIs For Mean Based on
Pooled StDev
Level N Mean StDev -----+---------+---------+---------+----
CPA 3 1.7567 0.4438 (-----------*-----------)
FPA 5 1.8300 0.0704 (---------*---------)
PA 5 1.9300 0.0436 (---------*--------)
PB 5 1.8020 0.1813 (---------*---------)
PC 2 1.8200 0.0283 (--------------*---------------)
SP 2 1.8100 0.0141 (---------------*--------------)
SU 5 1.7740 0.0904 (--------*---------)
U 5 1.8200 0.0557 (--------*---------)
-----+---------+---------+---------+----
1.65 1.80 1.95 2.10

Pooled StDev = 0.1580

Grouping Information Using Tukey Method

Treatment N Mean Grouping
PA 5 1.9300 A
FPA 5 1.8300 A
PC 2 1.8200 A
U 5 1.8200 A
SP 2 1.8100 A
PB 5 1.8020 A
SU 5 1.7740 A

CPA 3 1.7567 A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment
Individual confidence level = 99.71%

Two-Sample T-Test and CI: Cadmium Conc. (roots), Treatment

Two-sample T for Cadmium Conc. (roots)

Treatment	N	Mean	StDev	SE Mean
CPA	3	4.76	2.80	1.6
PA	5	1.766	0.398	0.18

Difference = mu (CPA) - mu (PA)
Estimate for difference: 2.99
95% CI for difference: (-4.01, 9.99)
T-Test of difference = 0 (vs not =): T-Value = 1.84 P-Value = 0.207 DF = 2

Two-Sample T-Test and CI: Cadmium Conc. (Foliage), Treatment

Two-sample T for Cadmium Conc. (Foliage)

Treatment	N	Mean	StDev	SE Mean
CPA	3	1.643	0.306	0.18
PA	5	1.386	0.354	0.16

Difference = mu (CPA) - mu (PA)
Estimate for difference: 0.257
95% CI for difference: (-0.348, 0.862)
T-Test of difference = 0 (vs not =): T-Value = 1.04 P-Value = 0.338 DF = 6
Both use Pooled StDev = 0.3385

One-way ANOVA: Lead versus Treatment

```
Source DF SS MS F P
Treatment 7 56.0 8.0 0.76 0.622
Error 24 251.3 10.5
Total 31 307.2

S = 3.236 R-Sq = 18.22% R-Sq(adj) = 0.00%

 Individual 95% CIs For Mean Based on
 Pooled StDev
Level N Mean StDev ---+---------+---------+---------+------
CPA 3 62.400 4.530 (------------*------------)
FPA 5 61.660 1.159 (---------*--------)
PA 5 62.080 2.223 (---------*---------)
PB 5 59.340 5.713 (---------*---------)
PC 2 61.350 1.202 (--------------*---------------)
SP 2 62.950 2.333 (---------------*---------------)
SU 5 59.230 1.501 (---------*---------)
U 5 62.080 3.108 (---------*---------)
 ---+---------+---------+---------+------
 57.0 60.0 63.0 66.0

Pooled StDev = 3.236


Grouping Information Using Tukey Method

Treatment N Mean Grouping
SP 2 62.950 A
CPA 3 62.400 A
U 5 62.080 A
PA 5 62.080 A
FPA 5 61.660 A
PC 2 61.350 A
PB 5 59.340 A
SU 5 59.230 A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
```

All Pairwise Comparisons among Levels of Treatment

Individual confidence level = 99.71%

One-way ANOVA: Lead versus Time (days)

Source DF SS MS F P
Time (days) 1 28.36 28.36 17.30 0.001
Error 18 29.51 1.64
Total 19 57.87

S = 1.280 R-Sq = 49.00% R-Sq(adj) = 46.17%

Individual 95% CIs For Mean Based on
Pooled StDev
Level N Mean StDev ------+---------+---------+---------+---
0 15 61.980 1.210 (----*-----)
211 5 59.230 1.501 (---------*---------)
------+---------+---------+---------+---
58.8 60.0 61.2 62.4

Pooled StDev = 1.280

Two-Sample T-Test and CI: Lead Conc. (roots), Treatment

Two-sample T for Lead Conc. (roots)

Treatment N Mean StDev SE Mean
CPA 3 29.9 16.8 9.7
PA 5 5.54 2.99 1.3

Difference = mu (CPA) - mu (PA)
Estimate for difference: 24.36
95% CI for difference: (6.53, 42.20)
T-Test of difference = 0 (vs not =): T-Value = 3.34 P-Value = 0.016 DF = 6
Both use Pooled StDev = 9.9809

Two-Sample T-Test and CI: Lead Conc. (Foliage), Treatment

```
Two-sample T for Lead Conc. (Foliage)

Treatment N Mean StDev SE Mean
CPA 3 5.040 0.950 0.55
PA 5 0.298 0.187 0.084


Difference = mu (CPA) - mu (PA)
Estimate for difference: 4.742
95% CI for difference: (3.725, 5.760)
T-Test of difference = 0 (vs not =): T-Value = 11.40 P-Value = 0.000 DF = 6
Both use Pooled StDev = 0.5695
```

ANOSIM Analysis

PRIMER 12/19/2014

Similarity

Create triangular similarity/distance matrix

Worksheet

File: C:\Documents and Settings\ebi\Desktop\santa susana\Santa Susanna 11-21-14.xls

Sample selection: 2-10

Variable selection: All

Parameters

Analyse between: Samples

Similarity measure: Bray Curtis

Standardise: No

Transform: Square root

Outputs

Worksheet: Sheet1

ANOSIM

Analysis of Similarities

Similarity Matrix

File: Sheet1

Data type: Similarities

Sample selection: All

One-way Analysis

Factor Values

Factor: treatment

SP

PC

U

Factor Groups

Sample treatment

SPc SP

SPb SP

SPa SP

PCc PC

PCb PC

PCa PC

Uc U

Ub U

Ua U

Global Test

Sample statistic (Global R): 0.613

Significance level of sample statistic: 1.4%

Number of permutations: 280 (All possible permutations)

Number of permuted statistics greater than or equal to Global R: 4

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SP, PC	0.889	10.	10	10	1
SP, U	0.963	10.	10	10	1
PC, U	0.074	40.	10	10	4